

Warnock proposals in trouble

By being dilatory, the British government has allowed its policy on in vitro fertilization to be undermined by an ordinary member of parliament. It should quickly set up a licensing committee.

THE predicted worst seems about to happen in the regulation of the new embryology in Britain. The six months since the appearance of the report of the Warnock committee (see *Nature* 310, 266; 1984) have over-strained the patience of some members of the House of Commons, whose clamour for legislation to ban this or that has now given way to the sport of drafting home-made and half-baked legislation. Mr Enoch Powell, the 1960s minister of health who shocked the pharmaceutical industry of the time by taking powers to let the British government buy round drug patents, and who has since found orthodox Conservative policy on social questions such as immigration so intolerably liberal that he has become an Ulster unionist, published last week the text of a blunderbuss bill. The effect would be to make all observation of human embryos a criminal offence, and to restrict the practice of *in vitro* fertilization to those cases in which the supposedly infertile woman had been named in advance by licensed physicians, who would have to have been given permission to go ahead by the Department of Health. If enacted, this bill would be much more restrictive than the Warnock proposals on the subject. The immediate anxiety is whether Mr Powell's bill can succeed.

Ordinarily, in British parliamentary procedure, private members' bills are not conspicuously successful. Much depends on the popular support they command. The most recent success for the procedure was the enactment (last year) of a bill to permit the censorship of video-films intended for use in people's homes, an illiberal measure so inconsistent with British law on other kinds of publications that the government, while sharing the populist opinion that what were called "video-nasties" should be banned, would not have wished to be seen to argue the case for extending censorship. The case of Mr Powell's bill is different. The government would obviously prefer to retain the initiative, introducing legislation of its own. Procedurally, it has many devices at its disposal for throwing Mr Powell's bill off course, from declining to provide parliamentary time for discussion to preemption, the introduction of a bill of its own. On this occasion, preemption would be the best strategy, but the government is in a fix because it seems not yet to have made up its mind what should be done, and because of the legal problems of drafting legislation that will satisfy popular opinion (hostility to surrogacy and the study of human embryos) without making criminal offences of existing practices. But the government will have to show its hand on Friday next week, 15 February, when the principles of the Powell bill are to be debated.

The danger is that Britain will be stampeded into over-hasty legislation which then, in the nature of things, will persist as law until some future government has the political courage to take on the alliance of interests opposed to novel practices in human embryology. The simplest and safest way out of this dilemma would be to forestall support for the Powell bill by enacting now what must be the essential ingredients of the system of regulation that will have to be established both for clinical practice and research. The generally agreed common denominator is a system for licensing both practitioners and laboratory researchers, and there is good reason why the government should go beyond the recommendations of the Warnock committee by requiring not merely the licensing of the people carrying out new procedures or research but also some kind of consideration of the necessity

for that work. In short, there should be a licensing committee whose function would be to license physicians offering established procedures (artificial insemination) as well as *in vitro* fertilization, to consider the merits of suggested scientific investigations (and to say yes or no) and also to consider on their merits proposals to carry out procedures that Warnock would have banned.

There is a precedent for some such arrangement, the Genetic Manipulation Advisory Group which had legal powers to sanction individual experiments in biotechnology and related fields. The system worked well, and served to satisfy both public anxiety that genetic manipulation would be attended by novel dangers and researchers, most of whom accepted that irritating delays were a necessary price to pay for public acceptance of a novel field. Exactly the same principles could be made to apply in human embryology. The issue is again that the British public (and many of its representatives in the House of Commons) have taken fright at some of the procedures that are now feasible, and that do indeed raise ethical and legal issues which are difficult and novel. But nobody can at this stage (the Warnock report notwithstanding) hope to know just what the problems are, let alone how best to balance the difficulties against the potential benefits, both in medical practice and research.

So much is clear from the response of the Medical Research Council to the Warnock recommendations, published at the end of last month (see page 424). The council accepts that there should be a licensing authority, and even suggests that it might set up a voluntary system of its own in advance of whatever emerges from the British government's brooding on the subject. But it also makes the telling point that the Warnock distinction between "research" (which would be allowed) and "new and untried methods of treatment", which would be disallowed, is nonsensical. The council also politely complains that the Warnock recommendation that inter-specific fertilization should be strictly forbidden could put an end to the present use of hamster ova as an assay for the motility of human sperm.

The plain truth, which all but the root-and-branch opponents of innovation in human embryology and the treatment of infertility must recognize, is that the present time is one at which the problems attending the new opportunities, both in medical practice and in research, are much more easily defined than are the benefits, which will become apparent only with the passage of time. That is why the government needs to buy not merely for itself but for the potential beneficiaries of the new techniques a breathing space in which mature consideration can inform permanent legislation. Setting up the licensing authority, backed by a legal requirement that would-be innovators should disclose their plans and wait for them to be approved, is the only way forward that makes sense. And that could be done quickly, perhaps even by next Friday.

The Law Society (see also page 424) has a series of more intricate points to make. "Consider first the interests of the child", it says, which is an appealing doctrine only for as long as the conventions of traditional society apply. But what about the real world in which the assumption that Mendelian genetics apply is distorted by the frequency of unconventional behaviour, non-marriage, for example? And is there not, in any case, a proper sense in which the interests of children in general may supervene over the interests of individual embryos? □



Big Foot's constituency

The search for mythical monsters in the United States is like that at Loch Ness.

THEY just don't make mythical creatures like they used to. For one thing, they used to come in a healthy mix of body sizes. But ever since the business fell into the hands of people with a special ability to raise money for expeditions to interesting places (invariably equipped with cameras whose lens caps show a special propensity for remaining in place at critical moments), little has been heard of leprechauns (small) or unicorns or centaurs (roughly average). Taking their place are the Loch Ness monster (monstrous), Yeti (abominable, if not large) and Big Foot (large feet, at least). Perhaps there is something especially appealing in the idea of modern science having missed big things. Certainly an expedition to hunt for mythical bacteria would not be a financial success. (But viruses are excellent candidates for "cryptoorganisms". If the proposed Reagan administration cut in NIH grants is carried out, virologists might consider making a direct appeal to the public for support of a hunt for "living fossils, mysterious half-dead, half-alive creatures that have colonized the Earth from its earliest days, invisible to us, ready at any moment to seize control of human or animal bodies to reproduce themselves". It might just work; sale of the movie rights could finance a new electron microscope.)

In reality, the correlation between ephemerality and size may be just one of those quirks of nature, akin to the indisputable fact that airplanes and ships mysteriously vanish but that trains never do. Regional pride may also matter, as with the competitors to the Loch Ness monster that have recently been championed. One of the odder recent manifestations of regional pride in defence of a local ephemeral best was in Washington State, where a public outcry followed the announcement by a former US Army Ranger that he was tired of all of those blurred photographs and footprints that, yes, might have been left by a big hominid but might, too, have been spots where a long-nosed dog laid down in the dirt, and that he was going to go out and shoot him a Big Foot.

Several indignant counties passed ordinances to outlaw the hunt. (They were also concerned about threats by a Big Foot conservation group is disrupt the hunt.) One local law made it a "gross misdemeanor" to kill a Big Foot with malice (one year in the county jail, \$5,000 fine, or both). The local chambers of commerce, which know a good thing when they see it, may have had a hand in the passage of the legislation. The thousands of outraged citizens who called the fish and game department to complain of the impending hunt are not so easily acquitted.

Local pride and a nose for publicity may also be the charitable explanation of the recent move by the commission overseeing matters related to Chesapeake Bay to take up the question of mounting a scientific investigation of "Chessie", the New World's answer to Nessie, as the Loch Ness version is known. (Actually, it is only one of the New World's answers; the Vermont Tourism Council would be sure to complain at any omission of reference to "Champ", which lives in Lake Champlain.) The potential for mischief here, however, is far greater than in the passage of ordinances declaring Big Foot out of season.

Chesapeake Bay, which provides fishermen in Maryland, Virginia and Delaware with a direct livelihood, and which indirectly supports much of the economy of the region, has become a model for studies of complex aquatic ecosystems and their interactions with man-made pollutants. Efforts to halt the deterioration of the bay depend greatly on developing a better understanding of these interactions, particularly the very complicated part played by non-point-source pollution, including agricultural run-off. It is sad to see the powers-that-be pursuing, or even contemplating, a will o' the wisp.

It is not clear just how Chessie came to be on the commission's agenda, though if the Big Foot outrage in Washington State is an indicator, it may have been the result of activism by devotees. Administrators should remember, though, that science is rarely

the right place to practise popular democracy. Those public officials who insist on seeing science as a place for special-interest-politics-as-usual should heed last year's Gallup poll which showed that only 18 per cent of US teenagers (usually a credulous bunch) said they believed in it, a sharp decline from 31 per cent in 1978. It is perhaps best not to mention how many said they believed in ghosts. Astrology (at 59 per cent) is obviously a less special interest. □

Oxford's indiscretion

Those who denied Mrs Margaret Thatcher an honorary degree should now count the cost.

FROM time to time, the University of Oxford distinguishes itself by means of a controversial ballot on a controversial topic. In 1937, for example, Oxford undergraduates voted for the proposition that they "would not fight for king and country", earning for their pains a certain notoriety and, by some accounts, helping to earn a short while later an opportunity to prove that they had not meant what they said. Last week, Oxford academics took up their ballot papers on the narrower issue of whether the British Prime Minister, Mrs Margaret Thatcher, should be awarded the honorary degree with which all previous British prime ministers who happen to have been Oxford graduates have been rewarded. The case against the university administration's plan was that the present British government has been beastly to British higher education and, latterly, towards research. Now, to their own considerable surprise, Oxford's academics find that the discontented have mustered two-thirds of the votes, that Mrs Thatcher has been snubbed and that the university's chancellor Lord Stockton (still better known as Mr Harold Macmillan), himself recently an oblique critic of the government, has taken them to task for discourtesy. Now, the worry is what the retribution will be.

Fair play will probably ensure that no ton of bricks falls on Oxford, or on universities in general. Direct public subvention of the universities is channelled through the University Grants Committee, unlikely to be much influenced by Oxford's show of defiance. Subventions from the research councils of academic research are similarly unlikely to be biased against what may be thought a wayward university, even though the Secretary of State for Education and Science, Sir Keith Joseph, is personally told of all proposals to make research grants above a value of £50,000. The delicate network of committees responsible for these distributions will no doubt meticulously avoid anything that seems like taking sides. The retribution will come in a different form, and will unhappily affect all British universities, not just Oxford.

The British government's policy towards higher education and academic research is generally acknowledged to be mistaken, short-sighted and potentially disastrous. The cuts in university budgets decreed four years ago were arbitrary and have since been enforced in needlessly arithmetical fashion. On research, the government has not until recently listened with anything like enough care to warnings that the system is so short of funds that the damage done would far outweigh the money saved by the economics demanded. Sir Keith Joseph's failure to deliver in full December's promise of extra money for the research councils and the grants committee, apparently an important factor in last week's vote at Oxford, can however be regarded in two ways — as a proof of muddle (which it was) and as a sign of repentance (which is probably also true). What voting Oxford academics last week overlooked is that British universities troubles have roots in the way the universities lost all their political friends in the late 1970s, after they had greeted the then government's statement of the academic money problem with a mixture of derision and indifference. In the past few months, there have been signs that the quarrel was about to be patched up. The danger is that a vote whose chief purpose seems to have been a snub will make it harder to bridge the conflict between academics and the rest of the world, and may even again inflame curiosity about universities' conduct of their own affairs that recently has been so unwelcome. □

US budget

Basic science still to grow, but less quickly

Washington

SCIENCE has emerged largely unscathed in President Reagan's proposed budget for fiscal year 1986. Although once again the major increases have been reserved for military research and development (see below), the federal programmes that support the bulk of scientific research, particularly basic research at universities, seem to have been granted a special dispensation from the draconian measures applied elsewhere in the non-military portions of the budget.

The large increases slated for the Department of Defense and the administration's refusal to consider a tax increase made it a foregone conclusion that this year's budget would not embody much improvement in the record federal deficit. The 1986 deficit is projected officially at \$180,000 million; even by 1988, the last year of Reagan's term, the deficit will be down only to \$144,000 million.

This is in spite of a series of severe cost-cutting measures: a 5 per cent pay cut for all federal civilian employees; a cut-off of government-subsidized student loans to families earning more than \$32,500 a year,

a cut-off for student grants at \$25,000 a year and a provision that no student can receive more than \$4,000 a year from public sources, loans or other subventions; a freeze on payments to physicians and hospitals under Medicare (which covers those eligible for social security retirement or disability payments) and a freeze on the number of persons covered by Medicaid (which is for those eligible for welfare payments); and a freeze on pensions for federal employees.

As always, the final shape of the budget for the financial year beginning on 1 October 1985, will be determined only after the details have been scrutinized by the responsible congressional committees and after Congress as a whole has had its say on the issues raised by the budget. The proposed cutbacks in social programmes and the increase of defence spending will be two major issues, the size of the deficit another.

Science has, however, taken its medicine (see below). While federal research and development is to increase by 12 per cent overall, the non-defence portion of that is actually due for a 4 per cent cut. Basic research is to receive a 1 per cent overall increase, behind the inflation rate. But researchers who rely upon the major federal grant-givers, the National Science Foundation (NSF) and the National Institutes of Health (NIH), have little to worry about. The 4 per cent cut is coming out of the technology-development programmes that the Reagan administration has never liked. And while there are to be no new construction projects beginning in 1986, neither will there be any cancellations of projects already under way.

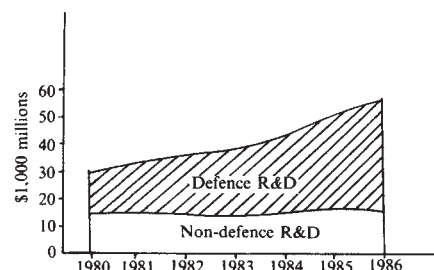
National Science Foundation

After two years of healthy increases in the NSF budget, this year's single-digit growth of 7 per cent may seem small. But that is still well ahead of inflation. NSF's engineering programme will receive the greatest percentage gain, 13 per cent; the founda-

tions's new engineer director, Erich Bloch, is pushing to expand the Engineering Research Centers programme at universities.

Mathematical and physical sciences are slated for an 8 per cent increase, as are biological sciences; earth sciences would get a 6 per cent rise. After initially resisting congressional enthusiasm and a National Science Board recommendation, the administration this year is proposing a major programme not only to improve access to supercomputers but actually to purchase machines and create supercomputer centres around the country at a cost of \$46 million.

Science education, which, along with all federal education programmes had been on the administration's hit-list until the



The growth in defence research and development during the Reagan years.

deteriorating state of the nation's schools became a campaign issue for President Reagan, will remain at the present level of \$83 million. Graduate fellowships will also remain at the present level (\$27 million within that total).

National Institutes of Health

Word leaked out last month about the administration's plans for dealing with the ever-expanding NIH budget, so there are no surprises in the official budget. Congress invariably tries to one-up the administration when it comes to NIH support; last year, Congress voted to give NIH extra money to increase new research grants each year from 5,000 to 6,500.

The administration, determined to hold the line, has ordered NIH to award only 5,000 this year, disposing of the excess funds by committing them to the future years of a portion of the three-year grants awarded now. The 5,000-grant limit would remain for 1986. The move would prevent annual increases of about \$300 million in

Losers for 1986

Washington

THE major losers in this year's science budget are not the major grant-making agencies. The Sea Grant programme of the National Oceanic and Atmospheric Administration (NOAA), disliked by science adviser George Keyworth, is due for elimination. NOAA's Great Lakes Environmental Laboratory would be shut down, as would the undersea research programme and the Coastal Zone Management grants. The National Bureau of Standards (NBS), like NOAA a part of the Department of Commerce, is also earmarked for cuts. The administration will try once again to terminate NBS's fire research and building research programmes; NBS would lose about \$17 million in operating funds overall, out of roughly \$120 million. Overall, the Commerce Department research and development budget is to fall by \$110 million, to \$271 million.

The US Geological Survey, part of the Department of the Interior, is due for a 2 per cent cut, to \$588 million in total obligations. Other losers are the Department of Transportation, the Veterans Administration and the Nuclear Regulatory Commission all of which face cuts of a few per cent in various small research programmes.

Stephen Budiansky

US research and development budget (\$ million)

	1984 actual	1985 estimated	1986 proposed
Defence-related	\$26,408	32,318	39,426
Health and Human Services (NIH)	4,836	5,472	5,159
Energy	(4,252)	(4,835)	(4,561)
NASA	4,642	4,805	4,712
NSF	2,877	3,506	3,730
Agriculture	1,203	1,354	1,447
EPA	868	940	882
All others	261	312	327
R&D facilities	2,104	2,248	1,915
Total	1,853	2,237	2,075
	45,052	53,195	59,673

NIH's budget. A major fight in Congress can be expected.

Agriculture

Hopes for a \$100 million competitive grants programme have evaporated. Last year, the Reagan administration managed against all odds to secure almost the full authorized level of \$50 million for this programme, the only alternative to the automatic formula-funding to the land-grant colleges and the agency's in-house Agricultural Research Service (ARS). With the farm bill up for renewal this year, there was talk of pressing an expanded programme that would become the major mechanism for supporting agricultural research. Instead, the administration is proposing to hold the programme at its present level of \$46 million. ARS is to be cut by 2 per cent, to \$485 million; formula funds will be held at last year's level of \$258 million.

Aeronautics and Space

The space station continues to dominate NASA's research and development programme, jumping to \$220 million from the present \$140 million. Physics and astronomy are due for a 10 per cent cut, planetary exploration for an 18 per cent increase. But these changes appear to reflect an overall decision to maintain previous commitments while not starting anything substantially new. Galileo and the Space Telescope are due for launch in 1986; work will continue as planned on the Venus Radar Mapper, the Mars Geoscience/Climatology Orbiter, the Gamma-Ray Obser-

vatory, the Explorer series of satellites and the Upper Atmosphere Research Satellite. Efforts to promote space commercialization will be boosted more than threefold, to \$40 million.

Energy

An overall 2 per cent cut is planned for the Department of Energy; following the well-worn Reagan Administration path, these cuts will come in magnetic fusion, solar and fossil programmes. But this time cuts are due in fission research as well, and even in basic research. The high-energy physics budget is to fall by \$35 million, to \$510 million; nuclear physics is to fall by 4 per cent, to \$173 million.

No new major facilities will be started in 1986, although there is a small request for funds to support research into future accelerator designs. The capital equipment request will provide "only the highest priority needs" for instrumentation at the Stanford Linear Collider and the Fermilab Tevatron accelerators now nearing completion. Preparatory work on the Continuous Electron Beam Accelerator Facility at Newport News, Virginia, is to continue.

Magnetic fusion is to take a major cut, however, from \$434 million to \$390 million. Fission research, which includes breeder reactor technology, will fall by 8 per cent. And alternative energy programmes are due for the annual Reagan attempt at virtual elimination.

Stephen Budiansky & Tim Beardsley

More for star wars

Washington

TRUE to its avowed policy of strengthening national defence, the administration's budget request for the Department of Defense is up by \$29,000 million, to \$323,000 million. The defence proposals can be expected to have a rough passage through Congress, however, and the department's 1986 budget plan might look rather different by the start of the fiscal year next October.

The administration is looking for a 22 per cent increase in defence-related research and development, bringing the total to \$39,400 million, or 50 per cent above the 1984 level (see graph). If the administration gets its way, the Defense Department will account for 65 per cent of total federal expenditure on research and development, estimated at \$60,000 million.

Basic research will be increased by 16 per cent to \$962 million, and there will be an "increased emphasis" on the development of a ballistic missile defence system — the Strategic Defense Initiative (SDI), better known as "Star Wars". Total proposed support for SDI in 1986 is £3,700 million; topics listed include space surveillance and target acquisition, directed energy and kinetic energy weapons battle management systems and system survivability.

Other major research and development projects are mentioned: the Small Intercontinental Ballistic Missile or Midgetman, with its hardened mobile launcher; the Trident II strategic missile; the MX missile, deployment of which is counted "essential"; and the Advanced Tactical Bomber.

Tim Beardsley

Expenditure on Strategic Defence Initiative (\$ million)

	1985	1986
Surveillance, acquisition, tracking and kill assessment	546	1,386
Directed-energy weapons	376	965
Kinetic-energy weapons*	256	860
Systems concepts and battle management	99	243
Survivability, lethality and support	112	258
Total	1,389	3,712

*This includes rail guns and new technology, as well as incorporating the high-velocity rocket programmes that had been part of the Army's old ABM programme for defending ICBM sites.

National Science Foundation

Board plans more active role

Washington

THE US National Science Board, the policymaking body of the National Science Foundation (NSF), last week took steps to increase its effectiveness in strategic planning. The hope is that, by wasting less time rubber-stamping grants that have already been approved in principle, the 24-member board will spend more time on identifying future scientific needs.

Constitutionally, the relationship between the board and NSF is anomalous, one that does not function well within the US government. In title supervisory, the board is composed of part-time people who cannot hope to keep on top of the full-timers at NSF and who cannot pretend to give them directions without causing trouble. Only rarely in the past three decades have the board chairman and the NSF director been eager for a symbiotic relationship, with the board functioning as a high-level lobbyist. But that the time may have come.

The science board, hitherto widely regarded as having been rather ineffectual, is required by law to approve all NSF programmes entailing a new commitment of \$2 million or more, or the spending of more than \$500,000 in one year. Despite inflation in science as elsewhere, these dollar amounts have not changed since 1968, so much time is now spent reviewing individual grants in the high-spending areas. In future, however, the board will delegate routine renewals to NSF director Erich Bloch; Congress will be asked to remove legal obstacles standing in the way.

The new initiative, which also involves a thorough shake-up of the science board's committee structure, has been instigated jointly by Bloch and Dr Roland Schmitt, chairman of the science board. Both men have brought big-business experience to their positions and the organizational changes are seen as a reflection of this. While the changes will not immediately affect most active scientists, prudent heads of research institutes will want to pay close attention to science board plans to give special support to deserving subject areas, according to Leonard Redecke, executive secretary to the NSF programmes council. A new committee of the science board will give increased prominence to the interests of minority groups.

There could yet be some troublesome legal obstacles, however. The NSF reauthorization bill would be the obvious way for Congress to make the changes to the National Science Foundation Act that NSF is now requesting but, because of a jurisdictional dispute between two Senate committees, NSF has not been reauthorized for the past four years. No early solution of that dispute is in prospect. □

Australian oceanography

Going to sea at last

Canberra

TWO research vessels now undergoing sea trials off Australia will make a big difference to the country's civilian research effort in the marine sciences, and so help to fill the large gaps in present knowledge of the enormous sections of the Indian, Pacific and Southern Oceans to which Australia lays claim.

Declaration of the 200-nautical-mile Australian Fishing Zone (AFZ) in 1979 made Australia responsible for approximately 7 million square kilometres of ocean, almost the same as the Australian land mass and second only to the US zone, which totals 7.8 million square kilometres.

In 1981, the Australian Marine Sciences and Technologies Advisory Committee (AMSTAC) painted a gloomy picture of the state of physical oceanography and marine geoscience. Its reports described

IMAGE
UNAVAILABLE
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REASONS

ORV *Franklin*

fragmented research and a general lack of coordination during the 1970s, stressing that the most obvious deficiency in the Australian programme was lack of access to deep-water research vessels.

The 53-metre *Soela* had served as a blue-water workhorse for the Commonwealth Scientific and Industrial Organization (CSIRO)'s Fisheries Division (at that time not yet split from the CSIRO Division of Oceanography), but Australia's small band of oceanographers had had to make do with the converted ocean-going tug *Sprightly* which, according to the AMSTAC report, barely met the minimum requirements for deep-water oceanographic research.

Keen to enhance Australia's credibility as the keeper of such a huge area, the Fraser government accepted a series of recommendations for the purchase or hire of a number of ocean-going ships totalling as many as six by 1986, but it was the end of 1984 before any of these craft hove into view.

A certain amount of space occasionally became available for civilian research on the Royal Australian Navy's 96-metre oceanographic vessel HMAS *Cook*, commissioned in 1980, but a great deal more work was judged by AMSTAC to be necessary in all fields. As a result, CSIRO will now have its own purpose-built blue-

water vessel and the Bureau of Mineral Resources, Geology and Geophysics (BMR) will charter one.

No new vessels are in prospect for the Department of Science's Antarctic Division, however, nor for the Australian Institute of Marine Science at Townsville, which concentrates on research into the Great Barrier Reef.

Last month, the CSIRO Division of Oceanography (now at its new home in Hobart, Tasmania) began putting the new ship, the Oceanographic Research Vessel (ORV) *Franklin*, through harbour and sea trials. Designed in West Germany, the 1,100-tonne 55-metre vessel will be operated as a national facility and, like the celebrated Franklin River in south-west Tasmania, is named after Sir John Franklin, Governor of Tasmania from 1837 to 1843 and recognized as the discoverer of the North-West Passage between Greenland and Alaska.

Built at a cost of \$A12 million, the *Franklin's* design complements rather than duplicates functions of other vessels used for fisheries research, geological exploration and Antarctic research, and so is not ice-strengthened or equipped with heavy trawling winches. The design, nevertheless, does embrace the needs of occasional and possibly inexperienced research teams working side by side on different projects. A total of twelve scientists may be accommodated and a crew of thirteen. With computer-controlled bow and stern thrusters, the *Franklin* can hold station under heavy conditions.

BMR, on the other hand, has chartered a specially-designed geoscientific research vessel for two years in the first instance. At 1,543 tonnes and 73 metres in length, RV *Rig Seismic* will be manned by a crew of fourteen and permits accommodation for up to twenty scientists for a maximum cruise duration of 80 days. In recent years, the BMR marine geosciences programme has included studies of Bass Strait, the Great Barrier Reef, and geoscience cruises in the south-west Pacific in partnership with New Zealand and the United States, to assist small island nations in assessing the geological framework and mineral potential of their offshore areas.

Early cruises planned for *Rig Seismic* include geophysical experiments and geological sampling of the Heard/McDonald Plateau near Kerguelen Island and cooperative projects with the Bundesanstalt für Geowissenschaften und Rohstoffe (West Germany) on cruises to the Lord Howe Rise, South Tasman Plateau and the Otway Basin. Later in the year, BMR will conduct two-ship crustal refraction experiments on the Exmouth Plateau in a cooperative exercise with RV *Robert D. Conrad* of the Lamont-Doherty Geological Observatory. **Jeffrey Sellar**

Canada baboon cruelty trial

Washington

A PHYSIOLOGIST and the chief veterinarian at the University of Western Ontario, Canada, are facing possible prosecution for cruelty to animals over an experiment in which a baboon was kept for several months in a restraining chair. The case is believed to be the first in Canada brought against researchers following complaints by an animal welfare group.



The experiment, by Dr Bernard Wolfe, was to examine dietary and hormonal influences on cholesterol and lipid metabolism. The veterinarian at the university who is also charged is Dr William Rapley. The experiment was supported by the Canadian Medical Research Council and the Ontario Heart and Stroke Fund; the protocol and the laboratory have been approved by the Canadian Council on Animal Care.

The complaint, filed by a group called LifeForce, followed a number of protests and break-ins at the university inspired by Wolfe's experiment, and some other experimental animals have been stolen. The complaint is made under a section of the Canadian criminal code which makes it an offence wilfully to cause unnecessary pain, suffering or injury to "an animal or bird". Whether the defendants will go to trial will be decided at a hearing before a provincial court judge on 15 February. The university will be meeting all of their legal costs.

The baboon in the experiment was fed a regular diet formulated for baboons; other nutrients were occasionally introduced by catheter into the gut. A university spokesman rejected the accusation of some protesters that the animal was totally immobilized; its head and legs were restrained and the animal could groom itself, though it was prevented from reaching the catheters. The baboon showed no obvious signs of distress, according to the spokesman, and spent much of its time watching television.

Tim Beardsley

Europe in space

Ministers to drive hard bargain

EUROPEAN science ministers agreed in principle at Rome last week to participate in the US project to place a permanent manned space station in space by 1992.

But any excitement that officials at the US National Aeronautics and Space Administration (NASA) may feel should be tempered with caution. For Europe is promising to drive a hard bargain, with what French sources describe as "the bitter experience of Spacelab" clearly in mind.

According to members of the European space technology centre, ESTEC, in the Netherlands, from which the Spacelab project was managed, Europe in negotiating was "not clever enough" in striking the deal that gave NASA two Spacelabs (pressurized microgravity and astronomical research modules) to fly on the Space Shuttle, in return for just one week of joint experiments.

The European investment in Spacelab is estimated at \$600-700 million, but the return has been small. Some European experiments continue "but we are at the mercy of what NASA committees decide". According to one British Spacelab user, US researchers are "screwing everything they can get" out of Spacelab, but rarely indicate its European origins. Hence the "bitterness" felt in France and a determination to do better with the space station.

Thus over the next two years the European Space Agency (ESA) will be negotiating terms of participation with NASA which will require:

- Participation in scientific planning.
- Guaranteed use of the space station even if Europe develops its own deep space communications system and manned access systems, and so does not need the whole US infrastructure.
- No "discrimination" against European users.
- Patents rights in results discovered.
- Fair costing.

These and other points must be "guaranteed" before Europe will make a final commitment to the space station, it is said at ESA.

Meanwhile, though, European ministers have agreed to enter "phase B" (detailed planning and costing) studies of a British Aerospace free-flying unmanned platform to which modular satellites may be locked, and which may fly in polar orbit or in an orbit somewhat above the space station; a German-Italian manned experiment module called Columbus (developed from Spacelab) to dock with the US space station; and the development of a large cryogenic engine (HM 60) to power a fifth series of Ariane conventional space launchers.

The ministers also agreed that further attention should be paid to means of pro-

viding independent European manned access to the space station. But there was no specific commitment to *Hermès*, a French mini-shuttle, or to *HOTOL*, a British concept of an air-breathing horizontal-take-off launcher, which the French science minister M. Hubert Curien jokingly described as "typical British right-angled thinking". Britain had developed the vertical take-off aircraft; inevitably, it was now talking of the horizontal take-off rocket.

Other than preliminary participation in the space station, ministers at Rome also agreed to an increased ESA space science programme, paid for by members' compulsory subscriptions to the agency. These will

British space agency

Centre seeking definition

BRITAIN has decided to establish a space agency, the British National Space Centre (BNSC), but it is not yet at all clear what the centre will do, what powers it will have, or how much it will have to spend. Britain may also spend more on *HOTOL*, the British Aerospace concept of a horizontal take-off spacecraft, which seems for the moment to be little more than a few notes on the back of an envelope and is, according to British Aerospace, "turn-of-the-century stuff".

As for BNSC, details will emerge "in a couple of months", says the Department of Trade and Industry, which shares with the Ministry of Defence the Royal Aircraft Establishment, Farnborough, where BNSC will "probably" be based.

Even this ghost has, however, been well-received by British industry, which has been pressing for better coordination of space policy, and the name alone no doubt impressed European partners in last week's ministerial space meeting in Rome. French and German ministers both commented favourably on Britain's apparently greater commitment to space.

Space scientists in Britain, however, will not be much impressed until it is decided whether BNSC will pick up the tab for membership of the European Space Agency (ESA). This is now £11.2 million a year and is paid by the Science and Engineering Research Council (SERC). British technology minister Geoffrey Pattie agreed in Rome to increase the sum by five per cent this year, and five per cent compound (and in real terms) for four years thereafter, so as to pay for an increased and more coherent ESA research programme ("Horizon 2000"). If SERC continued to pay this subscription, it would thus have to be finding another £4 million for space science in five years' time, which it can ill afford. But if Mr Pattie's BNSC paid the sum all would be well.

now rise by 5 per cent a year in real terms for five years, thus providing the basis for the "Horizon 2000" research programme worked out by ESA's science branch over the past year. Britain objected to agreeing the increase for ten years, however, as requested by ESA, and has limited agreement until 1989, when the sums must be renegotiated.

Nevertheless, according to ESA's scientific director, Roger Bonnet, the result was as good as could have been hoped (Britain had been talking of three per cent which, says ESA, would have killed the programme), and a start will now be made on "the first cornerstone" of Horizon 2000, the solar-terrestrial programme. This includes an observatory stabilized in the L_1 Lagrangian point between Earth and Sun, and a multi-craft space plasma physics mission.

Robert Walgate

This, however, raises the question of where BNSC will get its money from. Departments are already fighting their corners, the final question being how much power and influence they will be willing to cede to the new agency.

SERC, however, has a lot to offer: its Rutherford Appleton Laboratory has expertise in managing space projects, as do a number of university laboratories (such as Leicester and the Mullard Space Laboratory of the University of London), and there is some hope that these benefits could be recognized and used within BNSC in return for supporting the ESA subscription.

HOTOL seems even less cut and dried than the agency. Mr Pattie caused raised eyebrows in Rome with the concept of raising payloads by using air-breathing jets as far as the stratosphere and then firing the engines as rockets, using on-board oxidants thereafter.

There was some uncertainty this week as to how far the project was really developed. According to a spokesman for Rolls-Royce, whose experimental engine is reported as performing the air-breathing-to-oxidant switch, "I've no details. . . it has been built up so dramatically by British Aerospace and then by Mr Pattie. . . . We've only been asked to do some very minor research, which is really theory."

British Aerospace, on the other hand, claims that the propulsion system is "classified". Nevertheless the company admits that so far *HOTOL* is only a paper study combined with some model tests in a wind tunnel, "very much pre-proposal work — turn-of-the-century stuff".

The next stage is to secure funds for further development of the concept, which should eventually prove a cheaper launch system than either Ariane or the Space Shuttle, it is claimed.

Robert Walgate

International salaries

Britain backs pay rise at CERN

Paris

FRENCH officials were surprised and "extremely disappointed" last December when Britain failed to follow a French move to stop an increase in the salaries of staff at the European Organization for Nuclear Research (CERN) near Geneva.

"We have told the British Science and Engineering Council [SERC] several times that we were afraid of the consequences of Britain leaving CERN" (a possibility threatened by the existence of the Kendrew committee, due to report this spring on the value of Britain's continued membership of the organization) "and we thought this was an opportunity to reduce costs", said a French research ministry spokesman last week.

According to SERC, however, Britain failed to respond because the French delegation had not first tested the ground with the other CERN members, but sprang the proposal out of the blue (a fact France admits) and because the sums involved were negligible and could not solve SERC's problems.

According to the French ministry, however, agreement to the French proposals, which might have been won with British backing, could have saved enough to buy Britain a place in the European Synchrotron Radiation Source, in which SERC has expressed an interest but claimed it cannot afford to participate.

The figures work out like this. The CERN budget is at present SF 703 million, or £230 million a year. Of this, 36 per cent is straight salaries (48 per cent including allowances), or some £83 million. Britain's contribution to CERN, based on gross national product, is 16 per cent, so its contribution to straight CERN salaries is around £13 million. CERN was asking for a salary increase (to match inflation in Geneva) of 4.39 per cent — meaning £570,000 to SERC. France planned to halve the proposed increase, on the grounds that government-supported staff throughout Europe were receiving salary increases below inflation, and that international organizations such as CERN should also bear some of the burden. If Britain and other members had agreed with the French proposal, therefore, SERC would have saved some £285,000 a year. Whether this is negligible or not is a matter for debate. In the event, however, only the Netherlands supported France and the full increase requested by CERN was granted.

Whether CERN salaries can be held down in future, however, is another question. CERN staff complain that their salaries have been eroded by inflation for a number of years, and that this year — after the first CERN Nobel prizes — they were surely due at least constant payment. In Britain, and in France (whose border with Switzerland CERN straddles), CERN

salaries look astronomical. In gross terms, they generally reflect Swiss incomes, but in net terms they exceed the Swiss as CERN staff do not pay tax. Compared with other international institutions, CERN salaries are low, however: they are thought to come below those of the North Atlantic Treaty Organization, themselves below those of the European Commission in Brussels.

Also, according to a senior French physicist now working at CERN, there are great variations in circumstances at CERN. "But if you take the highest salaries here, they're double their equivalents in France." A French director of a laboratory of the Centre National de la Recherche Scientifi-

que, France's premier research council, earns some FF 19,000 (£1,700) a month net of tax, the physicist estimated, compared with FF 37,000 (£3,400) for the equivalent job at CERN.

The physicist saw this, however, as less a condemnation of CERN than a social problem in the salary ratio between visitors and CERN incumbents. "In the 1960s perhaps only 10 per cent of the physicists at CERN were visitors", he said, "but now the proportion is nearer 80 per cent. These people 'have a problem in living well' given the cost of living in the Geneva area, he said. However some visitors to CERN receive a cost-of-living bonus from their home countries. "The French receive nothing", said the CERN man, "the Italians and Germans a little. But the UK bonus is very good". **Robert Walgate**

UK industrial research

Bits and pieces of success

THE inconspicuous part of Britain's science establishment, that run by the Department of Trade and Industry, seems to be largely free from the constraints (mostly financial) that hamstringing the rest of it.

The department's science and technology report for 1983-84, published this week, shows an increase of support in these areas from £243 million in 1981-82 to £322 million last year. The buoyant growth is estimated (by last month's public expenditure survey) to have been continued in the present financial year to a peak (in real terms) of £383 million, whereafter spending is forecast to decline (in real terms).

This estimate seems certain, however, to be overtaken by events. The department's spending on space research and development (£63 million this year) may well increase as a consequence of last week's bullish British statement on the US space station at Rome (see p. 422).

At the same time, the department has been forced by the booming demand for grants for industrial research and development from industrial companies to halt its schemes in this area (from 1 November last) pending the outcome of a review due to be completed early in April. Some in industry fear the outcome will be more stringent criteria for making grants, the original purpose of which included that of tempting British industrial companies to appreciate the value of research.

The department's umbrella covers four substantial but now inconspicuous laboratories, including the National Physical Laboratory (with a budget of £19 million last year), the National Engineering Laboratory (£11 million), the Warren Spring Laboratory (£2.65 million) and the Laboratory of the Government Chemist, recently boasting of being at the cutting-edge of biotechnology in Britain, which appears to cost the Department of Trade and Industry virtually nothing, recovering its

costs by money transfers from other government departments.

The general impression of these laboratories offered in the report is unexciting; the Laboratory of the Government Chemist goes so far as to describe an important part of its programme (on the anaerobic digestion of waste materials, chiefly sewage) as "unglamorous". And the National Physical Laboratory, once (immediately after the First World War) the only British laboratory of importance, is described in terms suggesting a mundane interest in the minutiae of the application of metrology to the provision of secondary standards for British industry, although the laboratory is given credit for having developed a microwave system for measuring plasma temperature in the Joint European Torus, the thermonuclear apparatus being operated in Britain by a consortium of European governments.

Space research apart, information technology emerges as the department's chief interest in industrial development, not exclusively through the Alvey programme (which began spending its budget of £350 million last year). Last year's report tells of remotely controlled TV cameras and digital telephone exchanges developed for Malawi.

The record of how more than £300 million was spent last year is nevertheless more a catalogue of miscellaneous achievements, mostly small, than an account of a coherent strategy for the encouragement of industrial development. To some extent, the department's research and development programme is a prisoner of past commitments (as to the aircraft and textile industries), to other sections of industry (represented by "requirements boards") and of its resources (laboratories, people). But for an organization whose long-term objectives are economic, a more analytical account of how the money has been spent would be more appropriate. □

Warnock committee

UK agonizes on embryo research

GRATUITOUS advice on *in vitro* fertilization seems partly the explanation why the British government has not yet responded to the Warnock committee's proposals on *in vitro* fertilization and related matters. By 7 January, more than a hundred memoranda had been received by the Department of Health, since when both the Medical Research Council (MRC) and the Law Society (which represents lawyers allowed to deal directly with clients) have weighed in with advice.

The government's immediate difficulty is that the House of Commons is due on 15 February to give a second reading (from which approval in principle may follow) to a private member's bill introduced by Mr Enoch Powell, MP. The bill as published would restrict the practice of *in vitro* fertilization to women who had been identified in advance by their physicians, who would have a period of only four months in which to attempt a pregnancy, and would entirely outlaw scientific study of embryos. Ordinarily, the government would have sought to forestall such a bill with legislation of its own (see page 417).

Much of the new torrent of advice seems a reiteration of evidence already given to the Warnock committee, which reported in July last year after eighteen months of study. The Law Society's family law committee has however introduced a novel line of argument, in a memorandum published earlier this week, and has in particular advocated regulations of artificial insemination with donor sperm (AID) which goes further than the Warnock proposals.

Thus the committee argues for measures to avoid what it calls "genetic incest", for which purpose it asks that women who "knowingly and wilfully" are fertilized by the sperm of males to whom marriage would be prohibited should be held guilty of a criminal offence and that, in the same spirit, sperm donors should be identifiable in advance of the marriage of their unknown offspring. Similarly, it raises the question whether children should have access at the age of 18 to information about their genetic parents (as are adopted children under existing law).

On surrogacy, the Law Society would also go further than the Warnock committee by making it a criminal offence not merely to run a surrogacy agency but also to commission, or to provide, a rented womb. On the Warnock recommendation that study of human embryos should be permitted under licence up to 14 days, the Law Society complains that the Warnock report includes no principles by which research might be disallowed.

The lawyers say that "pressure to carry out research for its own sake (i.e. to satisfy curiosity, scientific or otherwise) is likely to become intense" and that the public interest requires that "limits have to be

fixed" before a licensing authority can be set up.

MRC's late response to the Warnock committee is almost at the opposite pole. Its memorandum welcomes the proposal that research should be undertaken only under licence but goes on to argue that because different embryos in different circumstances may develop at different rates, it would be better to define the end-point for study (the Warnock 14-day limit) by the appropriate stage of development.

The council also argues that legislation should not attempt a once-and-for-all

definition, but instead set up a licensing authority that would issue codes of practice that could be amended more easily than legislation proper.

The two documents probably illustrate as clearly as any the government's dilemma. There is evidently strong support in the House of Commons for the proposition, advocated by the Law Society, that it should be for Parliament to differentiate between the allowable and the disallowed. The lawyers say their guiding principle is the "best interests" of the child, regarded as an individual. MRC's case, explaining how research might lead to the understanding of congenital abnormalities, might be held to be in the best interests of children in general. □

European information technology

New Esprit deadline nears

Brussels

FOLLOWING the huge response to the first call for bids for the European Community's Esprit (information technology) programme, the second call for bids, whose deadline is 25 March, promises to be just as popular, but the European Commission's ability to cope with the demand will depend on a clear decision on the 1985 budget being reached before the summer.

A sum of 215 million European Currency Units (ECU; 1 ECU = £0.62) is to be allocated to the information technology research programme in 1985, compared with 180 million ECU 1984. But the rejection of the 1985 budget by the European Parliament last December means that staff recruitment for Esprit's task

force, needed to coordinate companies and researchers, has to be postponed. Commission officials expect that the present project management team's staff quota of 500 will have to be doubled by the end of 1985 and doubled again by 1986 in order to cope effectively.

For the first phase, 104 projects were selected out of 441 submitted for 50 per cent support by the European Commission over a 2-3.5 year period. In precompetitive research, microelectronics figured highest. The 27 contracts selected will receive 21 per cent of the total funds, the 23 office automation research contracts will receive 23 per cent of the funds available, while 30 per cent will go to 19 projects for computer-aided design and manufacture.

The 104 projects selected bring together 334 companies, 107 universities and 97 research institutes. Seventy-five per cent of contracts involve universities and research institutes, 70 per cent involve large companies, and 50 per cent include small and medium-sized companies employing fewer than 500 people.

Only 2 per cent of contracts have gone to non-European multinationals — ITT, IBM, Digital, AT&T and Bell, acting through their European subsidiaries. No Japanese companies applied.

Top participation came from West Germany, followed by France and Britain. General Electric Co. alone is participating in some 20 projects, followed by Plessey (13), ICL (eight) and British Telecom (six), while some British universities will collaborate with European industry on at least two projects each.

Karl-Heinz Narjes, who took over from Etienne Davignon as the new Research and Technology Commissioner in January, told the European Parliament's committee last week that the priority of research and development would now centre on exploiting industrially the results of basic research in Europe. He said he would also push for the creation of a European status for research workers.

Anna Lubinska

IT in Britain

As well as Esprit, British companies and researchers also have the option of applying to the Alvey programme, which last year awarded £350 million in grants to such collaborative projects. The Information Technology (IT) Skills Shortage Committee, however, set up to discuss the effects of the shortage of manpower on the future of IT in Britain, has concluded that both the education system and private sources of trained manpower "are barely keeping pace with the present demand". The committee recommends that companies take more responsibility for training employees as well as encouraging more government sponsorship of training schemes; it is estimated that demand for technologists and technicians will grow by 5-8 per cent over the next five years. One source of scientists for high technology industries in Britain may come from North America, where a recruitment consultant company's advertising campaign in ten US and Canadian cities resulted in 3,500 replies, mainly from British engineers and scientists. Of these, about 900 are said to be interested in jobs with British industry.

Maxine Clarke

Human and animal rights

SIR — The importance of animal research to human rights is frequently neglected. The atrocities and suffering in the Second World War led to several major agreements that embody an international consensus about basic human rights. These documents include the Universal Declaration of Human Rights (1948) and two related International Covenants (1966), as well as the Nuremberg Code (1949) on moral, ethical and legal principles for human experimentation, and the Helsinki Declaration of the World Medical Association (1964). The Universal Declaration states in Article 27 that "Everyone has the right to . . . share in scientific advancement and its benefits". The International Covenant on Economic, Social and Cultural Rights spells out, in Article 12.1, the more specific "right of everyone to the enjoyment of the highest obtainable standard of physical and mental health".

In seeking to enhance physical and mental health, biomedical and behavioural researchers often employ animal subjects. In part, such research is required by the Nuremberg Code and the Helsinki Declaration, as well as the codes of numerous national and international medical and scientific associations. Both the code and the declaration state (in the words of the Nuremberg Code) that human experiments "should be so designed and based on the results of animal experimentation and a knowledge of natural history of the . . . problem under study that the anticipated results will justify the performance of the experiments". That is, to safeguard human health and wellbeing, experimental procedures to be tried on humans should first have been tested on animals. While it may not be possible to observe this precaution in every case, experience has shown it to be a wise procedure wherever it is applicable. Similarly, many governments require that drugs and food products be tested on animals to assure their safety for human health.

In recent years, concern has increased regarding humane treatment of animals as pets, on farms and in agrofactories, in zoos, and in laboratories, and for the preservation of habitats necessary for the continued existence of endangered species. Scientific and professional groups observe stringent codes of ethics and humane procedures for research with animals. Laws and regulations have been formulated to deal with many aspects of treatment of animals, and some endangered species are protected by international treaties, but there is no formal recognition or general consensus concerning "animal rights".

Efforts to obtain humane treatment of animals are widely supported, but attempts to prevent the use of animals for research come into conflict with human rights, because interruption of research with animal subjects would result in the continu-

ation of grievous human suffering from physical and mental disorders that research could alleviate or prevent. Although some have claimed that all research relevant to human health and behaviour could be done with animal substitutes such as tissue cultures or computer simulations, this is clearly not true: if we could create satisfactory simulations for the human brain, we would already have solved the formidable problems of physical and mental health that face us on every side. Thus, concern for the human right to the highest obtainable standard of physical and mental health calls into question attempts to secure recognition for "animal rights" on a par with human rights.

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Determination of IQ

SIR — John Hartung's speculations (*Nature* 311, 515; 1984) that the shape of a person's nose determines his IQ are both offensive and inaccurate. The viscosity of moist air is less than that of dry air, while airway resistance is probably more influenced by the nasopharynx than the shape of the nose. Among all racial groups, the prevalence of lung disease is related to socio-economic factors. The increased incidence among blacks in the United States is therefore more likely to reflect their position in society than the shape of their noses. Dr Hartung's hypothesis appears to be a conscious or unconscious attempt to establish spurious grounds for white supremacy. His theory seems to me to be as unsavoury as those based on the shape of people's skulls which were used to justify the extermination of Jews and Gypsies in Hitler's Germany.

Dr Hartung's speculations on the effect of a subject's health on performance in IQ tests are, however, important since some of the hypotheses outlined are readily testable. It should be simple to prove in a controlled trial that allergic rhinitis impairs performance in IQ tests. The outcome would be of interest to all those involved in examinations. Furthermore, definite proof of such impairment would be of consequence in selecting employees for occupations in which a constant high level of alertness is required. Unlike Dr Hartung's experience, subjective and anecdotal evidence suggests no such impairment.

He also postulates that frequent absence from school is detrimental to performance in IQ tests. Two genetic diseases are cited, sickle cell anaemia and cystic fibrosis, as conditions that do not impair brain function. The first results in frequent thrombotic episodes in the brain, while the second is associated with failure to develop normally secondary to malabsorption. In con-

trast, physicians experienced in dealing with children requiring frequent admission to hospital, for instance those suffering from thalassaemia and renal failure requiring dialysis, feel that as a group these two examples are brighter than average.

Intelligent selection of suitable diseases that do not on *a priori* grounds affect brain function should allow the hypothesis that frequent absence from school impairs IQ compared with that of age-matched healthy siblings to be tested.

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Evolution dogma

SIR — In a review of the Kermacks' book on mammalian evolution (*Nature* 10 January, p.164), the following statement is described as dogmatic or old-fashioned: "Evolution is slow, continuous and imperceptible. The evolution of major taxa does not differ in any fundamental way from the evolution of one subspecies from another." The judicious modern view is implied to be that the evolution of major groups is rapid and involves unique genetic events. I invite your readers to compare Maynard Smith's statement¹ that "the genetic basis of species differences is similar in kind to that within species" with the title of W. Bateson's book² before deciding what is dogma, and who is old-fashioned.

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Benefits of DDT

SIR — The disaster in Bhopal has provoked a statement that "the process for manufacturing the new pesticide has already done more damage than DDT"¹. Various estimates have been made of the effects of DDT in India, including the lowering of infant mortality, prevention of 3.7 million cases of malaria in Bombay State alone during 3 years, and great increases in food production². Pal³ reported that in a 9-year period of use of DDT, the average span of life in India had increased to 47 years as compared with 32 years before the campaign against malaria. It thus seems likely that, ironically, many of the inhabitants of Bhopal actually owed their very existence to the use of DDT.

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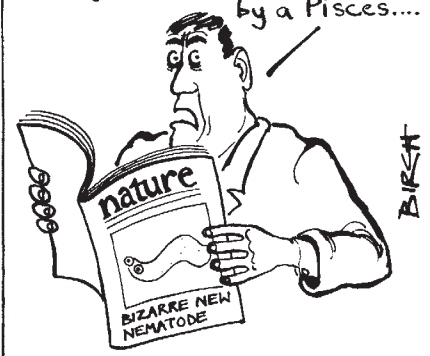
Astrology and cosmobiology

SIR — Reviewing our book *The Gemini Syndrome: A Scientific Evaluation of Astrology* (*Nature*, 15 November, p.219), H.J. Eysenck takes us to task for having “scandalously neglected” the work of the Gauquelins. Consequently, in our overall rejection of astrology, we are accused of carelessly discarding the baby with the bath water.

We agree with Eysenck regarding the attention deserved by the Gauquelins’ research. We recognize that it should be included in more detail with our treatment of astrology, though Gauquelin considers his work to be “cosmobiology” not astrology. The paperback edition of the book adds only a few pages of new material to the original version written in 1977–78, and we regret we could do little more than mention a few highlights and provide pointers to the recent literature.

On the other hand, we admit to a rather different perception from Eysenck’s of the

Of course, it's asking for trouble, having one's book reviewed by a Pisces....



value of astrology and his words of review appear to reveal a positive view rather than an objective one. To us, the Gauquelin correlations make no physical sense and are inconsistent with the body of natural empirical evidence. The correlations are weak, and it is not clear that they could not be spurious. In any case, the implication of a causal relationship is not justified on purely statistical grounds. There seems not a hint of a reasonable explanation for them. The successful replications are essentially only those by Gauquelin; Eysenck omits reference to the several careful studies that failed to confirm the effect.

The Gemini Syndrome was the first such book written by scientists to take a detailed look at the evidence rather than simply to declare astrology intuitively invalid. For our single sin of under-emphasis, our reviewer finds the entire book an unacceptable scientific evaluation of astrology. We have gained some understanding of how it feels to be the aforementioned baby.

Eysenck refers to the work of the Gauquelins as a “nugget of gold” amidst the rubbish of evidential support for astrology. In fact it seems the scientific

essay is not yet complete; we will be very surprised if the golden nugget does not turn out to be iron pyrite.

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SIR — It is surprising to see *Nature* including book reviews¹ that endorse superstition and pseudo-science. Eysenck is well known for his readiness to support controversial theories before there is any reliable evidence. An example is his support for “PSI” and parapsychology². It is interesting that Eysenck is often quoted by the tobacco industry on his critique of research correlating smoking with cancer.

Eysenck bases his positive assessment of astrology, or “cosmobiology” as he calls it, solely on the work of Françoise and Michel Gauquelin. He does not mention negative results obtained by others nor does he question the significance of the results.

The Gauquelins divide the sky into twelve sectors, the first beginning just over the eastern horizon. One of the claims mentioned by Eysenck is that the planet Mars appears in the sectors 1 and 4 at the birth of sports champions more often than for others (22 per cent instead of the expected 17 per cent). An attempt to replicate the experiment failed to yield positive results (13.5 per cent instead of 17 per cent)³. The results regained their significance only after the Gauquelins discarded a major portion of the data on the grounds that those came from “lesser sportsmen”. This raises the question as to whether the studies previously conducted by the Gauquelins were not similarly biased by *post hoc* selections.

Eysenck also fails to mention that the Belgian study referred to¹ had some major deficiencies. The sample size was small (535) and, more important, the sectors 9 and 10 appeared more often than the favoured sector 4. In addition, the Gauquelins were involved in this study as well, thus contradicting Eysenck’s claim of independence.

There is considerable doubt as to whether the work of Michel and Françoise Gauquelin is to be taken seriously in connection with astrology. On the contrary, there is every reason for responsible people to warn the public against being deceived by old-fashioned and modern astrologers. The book reviewed and the 186 signatories of the statement against astrology deserve our unrestrained support.

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Fever in autistics

SIR — Bryant’s recent review¹ of a book by Tinbergen and Tinbergen² was a timely reminder that our understanding of autism remains regrettably primitive. The theory originally espoused by Kanner³, that this distressing syndrome has its roots in the patient’s human environment, apparently still has its adherents, despite increasing evidence of an organic aetiology^{4–8} with hereditary origins⁹.

Possibly the strongest recent endorsement of the organic view comes from the widely observed (L. Wing, personal communication) but inadequately documented fever effect¹⁰. When autistics have a moderate fever, they invariably display dramatically more normal behavioural patterns¹⁰, including a greater desire or ability to communicate. It is difficult to see how these observations could be explained on the basis of environmental factors.

The effect appears to reach a maximum for fevers in the range 1.5–2.5°C (T. Rørsvol, personal communication). It seems unlikely that such a modest rise could appreciably influence the rates of either the metabolic processes or the molecular diffusion involved in neural function. But temperature changes of as little as 1°C can markedly alter the fluidity of membranes¹¹, such as those which form the synapses and the neurotransmitter-charged presynaptic vesicles.

An increase in the fluidity of these membranes would lower the vesicle-synapse fusion time, and thereby decrease the synaptic delay, which may well control what could be called the neural coherence length, which is a measure of the degree of inter-neurone cooperativity. Lower synaptic delays would increase this length, and one could speculate whether the autistic fever effect indicates that there is a connection between coherence in the behavioural sense and actual physical coherence at the neural level.

Equally intriguing is the possibility that autism stems from a neural lipid composition profile which departs from the ideal.

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The lack of progress in economics

from A.S. Eichner

It is possible for economics to become a science — if only the discipline would abandon its present practice of eschewing empirical tests of its theories in favour of 'formal' ones.

Of all the subfields within biology, the social sciences — economics among them — have made the least progress in developing a scientifically valid body of theory. This lack of progress has been attributed to the unpredictability of human behaviour, to the complexity and lack of permanence of social phenomena and to the difficulty of carrying out controlled experiments. At least insofar as economics is concerned, a different explanation can be offered — one which, if true, holds out greater hope that the principal obstacle to economics becoming a science can eventually be overcome.

An examination of economics as a discipline reveals that it is based on an epistemology, or method of establishing the validity of its knowledge claims, that runs counter to the norms of science. The prevalent view among economists is that formal (mathematical) proofs are not just helpful or indeed even necessary but also sufficient — and thus that empirical proofs can be dispensed with altogether. The result has been the development of a body of economic theory which is based entirely on axiomatic reasoning, without empirical support for the key propositions upon which the theory rests (P. Wiles, p. 67 in ref. 1). In contrast, those disciplines which have become sciences have done so because of the insistence that any knowledge claims, especially those which constitute the theoretical core of the discipline meet certain empirical tests.

Utility

The core of economic theory consists of four theoretical constructs which serve as the micro foundation for the dominant paradigm (A.S.E., p. 210 in ref. 1). These four theoretical constructs — indifference curves, isoquants, positively sloped supply curves and marginal physical product of capital curves — are derived from two more fundamental relationships. One is a 'utility' function representing the utility, or satisfaction, derived from the use of a given assortment of goods, $x_1, x_2, x_3, \dots, x_n$. This function is usually assumed to take the following general form:

$$U = U(x_1, x_2, x_3, \dots, x_n)$$

Certain 'first-order' conditions are then posited, namely, that the first derivative of the utility derived from any one of these

goods relative to that obtained from another is negative (the 'law' of diminishing marginal utility). From this first-order condition, a set of indifference curves is then derived representing the various combinations of goods which individuals consider to be of equal utility, along with a set of negatively sloped demand curves for each of those same goods indicating how the quantity demanded varies as the price of one good relative to another changes².

However, no evidence that such a utility function actually exists has ever been adduced — let alone any evidence that the 'first-order' conditions are satisfied³. All that is known is that households (consisting of one or more individuals) purchase goods of different types in varying quantities, with no means presently available to economists for determining the utility or satisfaction derived therefrom. Both the existence of the utility function and the postulated first-order conditions are said to derive from the logic of rational choice, based on the assumption that each individual seeks to 'maximize' his or her utility while having only a limited amount of income to spend (thereby facing a 'budget constraint'). Since the utility which the individual supposedly seeks to maximize as the necessary corollary to making a 'rational' choice cannot be observed and measured, the argument is tautological, without substantive content.

Production

The other basic relationship on which the prevailing microeconomic theory rests is a production function representing the different quantities of inputs, $z_1, z_2, z_3, \dots, z_n$, required to produce the output of the economic system. This function is usually assumed to take the following general form:

$$O = O(z_1, z_2, z_3, \dots, z_n)$$

with various first-order conditions again posited, in this case that the first derivative of the change in the quantity of one input required relative to the change in the quantity of another input, holding output constant, is negative (the 'law' of diminishing returns). From these first-order conditions are then derived (1) a set of isoquants, representing the different combinations of any two inputs which can be used to produce the same amount of output; (2) a set of positively sloped supply curves, in-

dicating how the quantity supplied varies with the price being charged, and (3) a marginal physical product curve for 'capital' (which is assumed to be one of the inputs).

The quantity of output produced, both by each industry and by the system as a whole, can be directly observed and measured. The production function, as usually specified by economists, is not therefore subject to the same criticism as the utility function. However, the capital which is assumed to be a homogenous physical input cannot be directly observed and measured, and thus the marginal physical product curve of capital, upon which the most widely accepted theory of income distribution is based, is no less metaphysical a theoretical construct (pp. 200-201, ref. 1).

Moreover, there is considerable empirical evidence contradicting the first-order conditions usually assumed in specifying the production function. What the evidence indicates is that the inputs used in the production process must be used in fairly fixed ratios to one another, with a shift from one set of these fixed combinations of inputs to another set occurring only when a new plant embodying the latest technology is constructed. Thus both the isoquants and the positively sloped supply curves usually postulated by economists lack empirical support, at least insofar as the non-agricultural sectors of the economy are concerned (A.S.E., pp 212-214 in ref 1).

Final demand

It is not for lack of an alternative that the present body of microeconomic theory is retained. Starting with the data available from input-output tables, it is possible to derive a separate production function for each industry that does not depend on any of the first-order conditions usually assumed in economic analysis. From the entire set of such production functions, it is then possible to explain both the quantities supplied by each industry and the corresponding set of relative prices, without having to assume separate demand and supply curves that are each a function of price. The values taken by output and price vectors depends on the level of final demand. By incorporating certain elements of what is known as post-Keynesian theory, it is then possible to explain the level of final

The misuse of mathematics in economics*

NEITHER the utility function $U = U(x)$ nor the production function $O = O(z)$ are functions in the mathematical meaning of that term. That is, no rule is provided by which, given $U(x)$, the value of U can actually be computed, and similarly for O and $O(z)$. We may be told that $U(x)$ and $O(z)$ are monotonically increasing and concave functions, but nothing more beyond such general statements. In consequence, there is not enough information to obtain an actual solution . . . By the very statement (or, rather, incomplete statement) of the problem, it is impossible to solve it! . . . This peculiar way of half-posing problems can be traced back to quite early times, but it was given its main impetus by Paul Samuelson in his very influential *Foundations of Economic Analysis* . . .

Why are problems of pure economic theory stated in such a peculiar, incomplete fashion? The reason is not far to seek. The utility function $U(x)$ is not given explicitly

because it *cannot* be given. It is an artificial construct of the theorist's mind and does not correspond to anything in the real world . . . Exactly the same is true of the production function $O(z)$. It is now more than a quarter of a century since Joan Robinson⁹ demonstrated that the quantity of capital, [one of the z 's] of that theory, is impossible to define consistently. Nonetheless, this very quantity still appears, with monotonous regularity, . . . in . . . innumerable papers on pure economic theory . . . All of this is *not* the application of mathematics to the economic problems of the real world. Rather, it is the application of highly precise and elaborate mathematics to an entirely imaginary and fanciful economic cloud cuckoo land. □

* An extract taken from J. Blatt, pp. 169–171 in ref. 1. The nomenclature of the mathematical functions is slightly changed to correspond with the convention used elsewhere in this article.

demand itself.

The growth of output per worker as one form of technical progress — to the extent it leads to a corresponding rise in real income — can be shown to determine, along with the growth of population, the long-term growth of final demand, while the variation in investment and other forms of discretionary expenditures can be shown to determine the cyclical movements of the economy around that trend line^{4,5}. This explanation for what happens at the industry and aggregate level, insofar as quantities supplied and relative prices are concerned, can then be supplemented by various 'behavioural' (as distinct from 'rationalist') models of what happens at the individual firm and household level. In this way, a complete and comprehensive alternative to the prevailing theories in economics can be developed—one that appears better able to meet the empirical tests necessary to establish economics as a scientific discipline^{6,7}.

Beliefs

The existence of this alternative nonetheless continues to be ignored by most economists, along with the evidence on which it is based, simply because it is not consistent with the four theoretical constructs that constitute the core of economic theory. Thus the prevailing theory in economics is based on an *a priori* set of beliefs rather than on any empirical evidence. In this respect, it is more akin to a religious credo than to any scientific body of knowledge. However, it is not just in refusing to accept the need for empirical

proof of its key propositions that the prevailing practice in economics runs counter to scientific norms. The violation of scientific norms also involves the following two fallacies:

(1) Acceptance of two, logically incompatible theories as being equally valid. This 'dualistic' fallacy characterizes not only the 'neoclassical synthesis', which combines the Keynesian macroeconomic theory with an irreconcilable pre-Keynesian microeconomic theory, but also the prevailing theories in 'pure' trade on the one hand and the models of international money and finance on the other.

(2) Construction of models to describe the market behaviour of economic systems which offer systems which differ in their fundamental characteristics from any market economy known to have ever existed and which, for that reason, are merely constructs of the mind unrelated to any external reality. This variant of the 'solipsistic' fallacy characterizes most of the work presently being done on 'general equilibrium models'.

These two misconceptions are in addition, of course, to the 'Cartesian' fallacy which underlies economic theory more generally — that is, the belief that formal proofs alone are sufficient. This Cartesian fallacy is reflected in the misuse which economists make of mathematics (J. Blatt, pp. 166–186 in ref. 1). Rather than serving as an aid to empirical research, mathematics is used to give economics the appearance, but only the appearance, of being a science. Mathematical relationships are posited, as in the case of the utility and

production functions specified above, but the unknowns remain precisely that — unknowns. They cannot be given a numerical value either because the parameters are unobservable or because the functions, being insufficiently specified, cannot be solved (see the accompanying quotation from J. Blatt). This misuse of mathematics is justified on the grounds that all that is required is that a proposition not be illogically deduced from whatever assumptions have been made. The proposition itself, along with the assumptions from which it is deduced, need have no empirical validity.

Validation

The fact that the prevailing practice in economics runs so counter to the norms of science suggests what must be done if economics is to ever become a science. Economists must come to accept as binding on themselves the rules which govern scientific work in general. At the very least, they must recognize the need to empirically validate the core body of economic theory along with the need to replace any theoretical constructs which cannot meet that test.

It is doubtful, however, that so radical a change will occur without considerable pressure from outside the discipline. The same mechanisms which, in other fields, lead to the reinforcement of scientific norms—the system of graduate training, the appointment and tenuring of faculty, the refereeing of journal articles and the peer review of research proposals—instead act to preserve the core body of non-scientific theory in economics (A.S.E., pp. 225–235 in ref. 2; P.E. Earl, pp. 90–125 in ref. 1). It may therefore require the censure, or at least the strong protest, of the scientific community to force a change in the prevailing practice among economists. The protest would be immediate if creationists and others falsely claiming to be scientists were being similarly rewarded with grants from the National Science Foundation and with prizes from the Nobel Committee. □

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How special is special relativity?

A neat new measurement confirms that moving clocks appear to run slow, and by the amount that special relativity predicts (with an accuracy of one part in 25,000).

SINCE 1905, the most robust torrent of correspondence between *Nature* and its readers has largely served to illustrate the widespread disbelief in Einstein's proposition that the speed of an apparently-moving clock will appear to be less than that of an identical clock apparently at rest. Like the general unwillingness to believe that the present mixture of species on the surface of the Earth in neither the consequence of an accident nor of a grand design but something in between (evolution by natural selection), or the widespread conviction that there can be no physical means by which two electrons (or, in general, fermions) can be prevented from occupying the same physical state, disbelief in time dilation is common. It should be banished by the results of an experiment now reported from the University of Aarhus (Denmark) demonstrating that time dilation is a fact, with an uncertainty of one part in 25,000. The estimated accuracy is less telling than the neatness.

There is a sense in which the demonstration of time dilation predates special relativity. The Michelson-Morley experiment in 1887, devised as a means of measuring the Earth's velocity relative to the aether but which surprisingly yielded a null result, can be interpreted in retrospect as a demonstration of time dilation. What Kaivola *et al.* (*Phys. Rev. Lett.* **54**, 255; 1985) have done is to follow through a proposal by J.L.Hall, from the US National Bureau of Standards at Boulder, Colorado, that the use of tunable lasers stabilized by being coupled together in a heterodyne system (in which their frequency difference or beat frequency can be measured) would allow accurate measurement of the time dilation of special relativity. (See J.L.Hall in *Atomic Physics 7*, eds D.Kleppner and F.M.Pipkin, 267-296; Plenum, New York, 1981.) With Brillet, Hall has the distinction of having used modern techniques to reproduce the result of the Michelson-Morley experiment to one part in 10^{15} .

Being a null experiment, Michelson-Morley has the disadvantage of being open to several alternative explanations among which time dilation is only one. Direct measurements of time dilation must be more direct. The simple way of attaining high accuracy is to seek to accumulate the effects of time dilation over a long period of time, which may be done by means of experiments recording the time told by clocks carried in Earth satellites, the precision of which should in principle ac-

cumulate as time goes by. The snags are that the motion of a satellite in the Earth's gravitational field is not a simple problem in special relativity and that, in any case, the strictly relativistic effect of time dilation is second-order compared with the predominant first-order and classical Doppler effect. Another way of putting it is to say that the strictly relativistic time dilation, being proportional to $(v/c)^2$, should show up even when the first-order effect is zero, or when the moving clock is moving transversely to the observer. That is why everybody is keen to measure time dilation directly, instantaneously so to speak, with a system in which there is a well-defined relative velocity and in which the time-dilation appears continuously, as a frequency difference.

One of the most interesting experiments of this kind so far is that reported almost a decade ago from CERN, when a beam of muons in a circulating storage ring was measured with previously unknown precision (Bailey, J., *et al.* *Nature* **268**, 301; 1977). The objective was to measure the half-life against radioactive decay of the circulating mesons, to relate this to their supposedly much shorter average lifetime in a state of rest and to demonstrate that the result was that predicted by special relativity. The outcome, a triumph at the time, was accurate to one part in 1,000.

Kaivola *et al.* have done better by a factor of more than 20 by a quite different technique. In place of moving clocks such as are provided by the natural decay of muons, they use atomic beams of neutral neon in which the clocks are the transition frequencies between defined electronic excitation levels. Specifically, they are concerned with the transition between the $3s$ and $4d$ electronic levels by way of the almost exactly intermediate $3p$ level. In reality, the lower state, being symmetrical, has a single energy, but the intermediate state is a complex of six (allowing for the interaction between an unsymmetrical electronic orbit and the unsymmetrical almost-completed shell formed by detaching one electron). By good chance, one state in this complex falls almost exactly half-way between the lower and upper states — the difference is roughly 3000 MHz.

The trick in the experiment is to lock the frequencies of two separate lasers to the two-photon transitions from the $3s$ to the $4d$ states of neon atoms which are respectively at rest (in a motionless cell) and moving in an atomic beam with an energy of

120 keV. In the fixed cell, the two-photon transition can be made to resonate strongly because there is a group of intermediate states. In the atomic beam, the second laser is made to feed an optical cavity aligned along the direction of the beam, in which case the transition frequency from the lower state to the lowest of the intermediate states matches the frequency of the laser beam in one direction, and the final excitation frequency matches the laser frequency in the opposite direction. The laser is tuned until the system is in resonance, but it is not then necessary to measure the actual velocity of the atomic beam. The result of the experiment is simply a measurement of the frequency difference between the two lasers, locked in frequency respectively to the fixed cell and the atomic beam.

The interest of the result is that it is precisely what would be expected from special relativity, not merely in the numerical result but in the way in which an atomic beam with two closely separated transition frequencies should be able to absorb the first photon from one directionality of the standing-wave radiation field in which it moves, and the second photon from the oppositely directed field. Qualitatively as well as numerically, the prediction is nicely confirmed.

So what happens next? The authors of this neat measurement say, with an air of resignation, that their equipment cannot "easily be improved". They think the next step will be the use of stabilized heavy-ion storage rings as a source of neon atoms of well-defined energy (in which case more accurate measurements of the transition frequencies will be needed). But will the doubters of special relativity wait that long?

Those who cannot should first read the article by Hall (above), which is mostly an eloquent account of what measurements may be done with stabilized lasers but which incidentally gives a clear account of complicated argument due to Mansouri and Sexl of how special relativity as we know it may be regarded as but one of a triply parametric series of theories distinguished by Einstein's recipe for synchronizing clocks. From this point of view, verifying special relativity is not a matter of demonstrating something special, that the velocity of light is the same in all directions, for example, but of making measurements to distinguish between different theories — and finding time dilation to be what Lorentz and Fitzgerald independently first showed. **John Maddox**

Astrophysics

Softening of neutron stars

from Philip J. Siemens

ADVANCES in particle physics in the past decade have not only clarified our view of the structure of matter and vacuum at very tiny distances but have also revolutionized our picture of the cosmos. The most striking implications have been drawn from studying the first microsecond of the Universe, before the fundamental quanta—quarks and gluons—coalesced into the heavy neutrons and protons which, with the lighter electrons and photons, make up most of the matter in our world today. In a recent paper¹, astrophysicists from Copenhagen, Krakow and Illinois extend these implications to include current dynamic processes in our Universe. They conclude that supernovae are more likely to produce black holes than had been thought, and less likely to produce the more readily observed pulsars or neutron stars. Indeed, pulsars are observed in only a few supernova remnants².

Kutschera, Pethick and Ravenhall trace the paucity of neutron stars to the inability of their material to resist the crushing force of gravity¹. A neutron star is formed when a star about ten times as massive as our Sun has burned all its nuclear fuel. Without the outward pressure of heat and light from nuclear power to counteract the inward pull of its own gravity, the star collapses until the neutrons and protons in its interior bump into each other. Their collisions produce a shock-wave of pressure which travels outwards through the star, blowing the surrounding material into space in a spectacular explosion known as a supernova. About a tenth of the star's mass is left in the middle of the explosion, within a radius of about ten kilometres. Whether this remnant becomes a pulsar or a black hole depends on its mass and on the strength of the material of which it is made. If the neutrons and protons are able to resist being crushed into each other by the enormous gravitational pressure, the remnant will become a neutron star (eventually, nearly all the protons absorb electrons to become neutrons), its rapid rotation giving rise to the pulses of radiation by which the pulsar is identified. If gravity wins, the remnant keeps on collapsing until its gravity becomes so intense that it becomes a black hole, cut off from the rest of the universe. For the heaviest remnants, gravity wins; for remnants as light as our Sun, the neutron star is stable. The new study places the dividing line at about 1.5 solar masses compared to about 2 estimated from previous work.

The conclusion that neutron-star matter is softer than previously believed follows from a new picture of what happens when neutrons are crushed together under pressure. This, in turn, is based on a radical

concept of neutrons. In older theories, a neutron is either taken as a fundamental, indivisible particle or (more recently) as a group of three such particles (quarks), bound together by strong interaction fields of force (gluons). In each case the particles are specified while their interactions are described by wave-fields of force around them. In the new picture, the fields are the main 'objects', while the particles are solitons or solitary waves, representing kinks in the field. These kinks arise as localized singularities when the field, which extends over all space, is thought of as a mapping of each point in space and time into a manifold characterizing the field. This manifold consists of all possible combinations or various mixtures of the different kinds of 'flavours' of field. The solitons are places where the fields change abruptly. Various types of solitons are possible, depending on the topology of the manifold of fields. The neutron-star computation uses a theory with four fundamental fields, known as the chiral field theory; together with its equations of motion and the identification of its singularities as neutrons, protons and other particles, this theory is known as the 'chiral soliton model'. It was invented over 25 years ago by T.H.R. Skyrme^{3,4} and chiral solitons are now sometimes known

as 'skyrmions'.

Considered a mere curiosity when it was introduced, the chiral soliton model has drawn much attention since Witten⁵ connected it to the modern gauge-field theory of quarks and gluons, known as quantum chromodynamics or QCD. This has many more fields than the chiral soliton model and has its own elementary particles as well; nevertheless, Witten showed that Skyrme's model is a correct approximation to QCD for experiments which do not probe too deeply into the interior of the neutron. Unfortunately, another approximation has to be made to obtain Skyrme's model, which corresponds to setting the pi meson's mass to zero. True pi mesons, the primary agents that bind atomic nuclei together, are, however, not massless.

Many nuclear theorists are working to improve the model so that it accounts correctly for the attractive forces that bind nuclei as well as the repulsion that stabilizes neutron stars. The computation of neutron-star matter using skyrmions shows how to apply QCD to the Universe's largest nuclei, neutron stars. It is an important step towards an understanding of the structure of atomic nuclei. □

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Primates

First fossil tarsier from Africa

from R. D. Martin

IN THE evolutionary tree of primates, the tarsiers (*Tarsius* spp.) occupy a special position. These diminutive, nocturnal primates, weighing approximately 150 g, are generally thought to be intermediate between the other prosimian primates (lemurs and lorises) and the simian primates (monkeys, apes and man). There is, therefore, considerable interest in their ancestry and in the relationship between surviving and fossil tarsiers. In that respect, a fossil described on page 475 of this issue comes as a major surprise to primate palaeontologists.

Hubrecht, the father of modern comparative embryology, noted in 1908 that tarsiers share with simians a particular form of invasive (haemochorial) placentation. Ever since, evidence has gradually accumulated to suggest that tarsiers and simians share a specific common ancestor. Living tarsiers share with living simians a whole suite of morphological similarities, including features of the brain, major sense organs and skull structure. It has also been found that tarsiers resemble simians in

chromosomal and biochemical terms.

In a major review of the morphological evidence in 1981, Pocock noted that whereas lemurs and lorises, like other mammals, have an area of naked, moist skin (the rhinarium) surrounding the nostrils, tarsiers and simians (alone among land-living mammals) have lost all visible trace of this structure; their nostrils are surrounded by hairy skin and there is a true face. Pocock suggested a fundamental division of the primates into Strepsirhini (lemurs + lorises) and Haplorhini (tarsiers + simians). Although it is still not universally accepted that tarsiers are the closest living relatives of simians, the consensus of opinion is increasingly moving in that direction and numerous authors have found it convenient to split the living primates, at least, into 'strepsirhines' and 'haplorhines'.

Despite their particularly interesting intermediate position in the primate evolutionary tree, tarsiers are still quite poorly known, largely because of their very limited geographical distribution. The three

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Tarsiers, our neglected relatives. (Zoological Society of London)

closely-related species are all confined to islands of south-east Asia — *Tarsius bancanus* in Borneo, Java and Sumatra, *T. spectrum* in Sulawesi and *T. syrichta* in the Philippines. During the early Tertiary, however, relatives of the modern tarsiers were far more widespread. Fossil tarsioids (now commonly allocated to the single family Omomyidae) have been documented extensively from North America and Europe, and very fragmentary fossil evidence has recently been reported from Asia, though the known material is essentially limited to the Eocene. In North America, tarsioids may have survived into the early Miocene, but in Eurasia there is no record of tarsioids beyond the earliest Oligocene. Thus, in the Old World there is a yawning gap in the fossil record between the Eocene tarsioids and the few living tarsier species. Until now, it has been widely accepted that modern tarsiers are most closely related to the best-represented group of European Eocene tarsioids (subfamily Microchoerinae) and on dental grounds *Tarsius* has been specifically linked to the microchoerine genus *Pseudoloris*. Accordingly, modern tarsiers have plausibly been suggested to be survivors of a once widespread Eurasian tarsioid stock.

This relatively straightforward, if very incomplete, picture of tarsioid relationships must now be re-examined in the light of the discovery, reported by E.L. Simons and T.M. Brown on page 475, of an apparent tarsioid, *Afrotarsius chatrathi*, from the African continent. It comes from the well-known Fayum deposits of Egypt, which have already yielded an important series of early simian fossils of Oligocene age and, more recently, the first marsupial fossil from Africa. The single reported specimen of *Afrotarsius* is tantalizingly incomplete, consisting only of a partial lower jaw ramus bearing five damaged cheek teeth. Living tarsiers and fossil tarsioids of the subfamily Microchoerinae are specifically distinguished by peculiarities of the lower anterior dentition (incisors and canines) but, unfortunately, these teeth are lacking from the new specimen. Nevertheless, the molar teeth of *Afrotarsius* closely resemble those of known tarsioids and are strikingly similar to those of the living *Tarsius*.

Tarsiers are unusual among living primates in that they have retained the paraconid cusp on the lower molars, whereas this cusp apparently disappeared

early in the evolution of the other extant primate groups. Fossil tarsioids have also retained the paraconid cusp and fine details thereof link *Afrotarsius* to *Pseudoloris* and, especially, to *Tarsius*. We are thus faced with the puzzling situation that the closest known relative of *Tarsius*, in dental terms, comes not from Eurasian deposits but from Africa. Furthermore, the close dental resemblance between *Afrotarsius* and *Tarsius* throws some doubt on recent suggestions that tarsiers may be more closely related to simians than to fossil tarsioids.

Afrotarsius is of considerable interest in its own right as a likely addition to the primate fauna of Fayum (which is the only early Tertiary fossil site from Africa to yield substantial primate remains). Dental dimensions indicate that *Afrotarsius* was even smaller than *Tarsius*, with a body weight of perhaps 90 g, close to the modern dwarf bushbaby (*Galago demidovii*). Although the modern African primate fauna contains both simians (Old World monkeys) and prosimians (bushbabies and pottos), somewhat surprisingly only simian fossils had been found in the Fayum deposits before *Afrotarsius* was unearthed. It is even more surprising, however, that the first prosimian fossil reported from the

Fayum should turn out to be a tarsioid rather than an early strepsirrhine. Thus, as with many new fossil discoveries, *Afrotarsius* poses more questions than it provides answers.

Because of the extremely fragmentary nature of the *Afrotarsius* specimen, little new light is shed on the strepsirrhine/haplorhine dichotomy, although it provides reinforcement of the view that the closest known relatives of the tarsiers are indeed the early Tertiary fossil tarsioids. The distinction between strepsirrhines and haplorhines is largely based on evidence from living primates, and many primate palaeontologists have resisted the application of this distinction to fossil primates, most of which are known exclusively from dental remains. It should, therefore, not escape notice that in their account of *Afrotarsius*, Simons and Brown explicitly refer to the "suborder Haplorhini", denoting a significant shift away from Simons's previous classification, which employed the traditional suborders Prosimii and Anthropoidea. □

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EISCAT

Coherence from incoherence

from Henry Rishbeth

A RECENT issue of the *Journal of Atmospheric and Terrestrial Physics* demonstrates how hard the scientific community is working on the data flowing from the European Incoherent Scatter (EISCAT) facility, and the advanced nature of the system¹.

UHF transmissions from EISCAT began in August 1981 but, owing to various problems, the system did not become fully reliable until the late summer of 1983. Since then the 933 MHz UHF transmitter at Tromsø has accumulated 2,170 hours of operation during the year ending 30 September 1984, comparable to the original annual target. This has been achieved despite a few weeks' break to implement an improved operating system, and the need at times to interrupt UHF operations while the more powerful 224 MHz VHF transmitter is being installed.

Half the total operating time is devoted to 'Common Programmes', comprising runs of 24–36 hours in standard modes, which will continue over a solar cycle. Through the International Geophysical Calendar, these programmes are co-ordinated with operations of the French incoherent scatter radar at St. Santin and the Western Hemisphere radars at Jicamarca (Peru), Arecibo (Puerto Rico), Millstone Hill (Massachusetts) and Søndreström (Greenland). The other half of the observing time is devoted to 'Special Pro-

grammes' of the six EISCAT associates, which are the national scientific organizations of Finland, France, West Germany, Norway, Sweden and the United Kingdom. The flow of data from EISCAT is stimulating new thought about the physics of the incoherent scatter process and the techniques for extracting physical parameters from the data; early results have been described in these columns and elsewhere^{2–4}.

EISCAT has produced the first direct evidence for anisotropic ion temperature in the auroral F-region at about 300 km height. At times, marked differences are found between the ion temperatures simultaneously measured at the three UHF receiving sites, implying that the ion temperature perpendicular to the geomagnetic field is up to 50 per cent greater than the field-parallel temperature. The anisotropy occurs when the ionospheric electric field is large and variable, and appears to result from frictional heating of the rapidly-drifting ions by collisions with the neutral air.

Middle atmosphere science is benefiting from EISCAT. Multipulse groups and coded pulses have been used to study sporadic E layers with very high resolution, 450 m in height and 10 s in time. The data show that layers only 1 km thick are sometimes embedded in broader regions of auroral ionization. These experiments make pos-

sible a new attack on the physics of sporadic E. In the course of one experiment, the occurrence of a polar cap absorption event enabled electron concentrations to be measured at heights down to about 70 km. D-region studies are giving new information on how the electron distribution is related to the absorption of HF radio waves, an important factor in high-altitude radio communications. Other experiments have measured winds and investigated the ratio of negative ion to electron concentrations at heights of 65–85 km, where few ground-based techniques are available.

The combination of EISCAT and ground-based Fabry-Perot interferometers — sometimes valuably complemented by data from satellites such as Dynamics Explorer — provides a powerful means of studying upper atmosphere dynamics at heights of 250–300 km, in particular the response to heating by electric currents or energetic particles. The interferometers measure the temperature and line-of-sight velocity of the neutral air, using the O(I) 630 nm emission, and sometimes of the ions, using the O(II) 732 nm emission. So far, this has been done at EISCAT sites only during hours of darkness and (of course) when the sky is clear. The observations clearly show that at times of geomagnetic disturbance, the ion flow speeds up and the pattern expands towards lower latitudes. The zonal neutral-air wind responds rapidly to individual disturbances, but the meridional wind responds more slowly, apparently being determined by larger-scale phenomena.

Several good sets of data are available from the optical observations made at Kiruna, close to EISCAT, and at Spitzbergen, 10° north of the EISCAT transmitter. In one experiment, the combination of optical O(II) measurements from Spitzbergen and EISCAT data from Tromsø led to the conclusion that there was an unexpected strong downward component of ion motion. A general conclusion seems to be that vertical motions of ions and neutral air are of greater importance at high latitudes than at mid-latitudes. EISCAT has also been used in conjunction with television studies of aurora, which show that visual arcs, less than one kilometre thick in the magnetic north-south direction, are embedded in regions of ionization some tens of kilometres in thickness. Simultaneous measurements of the E-region ionization produced by energetic electrons, by EISCAT and by particle detectors on the geostationary satellite GEOS-2, have made it possible to study the movement of the 'footprint' in the ionosphere of the magnetic field line on which GEOS-2 is situated. Other EISCAT programmes, sometimes run in conjunction with passes of suitable satellites such as HILAT, ARCAD-3, and the NNSS series, are designed to investigate kilometre-scale structure of the auroral F-region. These studies have a bearing on practical problems of the

propagation of HF, VHF and UHF radio waves at high latitudes.

Investigating the large-scale circulation of ionospheric plasma at high latitudes, which is driven by the interaction of the solar wind and the magnetosphere, has always been a major scientific objective of EISCAT. Maps showing the plasma velocity over a range of latitudes, for 24 hours at a time, can now be routinely produced by a Common Programme. In October 1984, EISCAT data were directly compared with interplanetary magnetic field data from the AMPTE-UKS satellite⁵, situated in the solar wind on the dayside of the Earth; the interplanetary field data were displayed in real time at the Rutherford Appleton Laboratory, to which were relayed ionospheric plasma velocities measured 1,000 km north of Tromsø. Features of immediate interest were the increases in plasma velocity that followed southward turnings of the interplanetary field, and the occurrence of short-lived northward surges of plasma, possibly associated with 'flux transfer events' in which the interplanetary magnetic field connects with terrestrial field lines⁶.

An important part of EISCAT's programme is the cooperation with the West German HF 'ionospheric heater', which can radiate several megawatts into the ionosphere above Tromsø. Using the ionosphere as a plasma laboratory in which man, as well as nature, can perform a variety of experiments (with EISCAT as a 'plasma diagnostic'), much is being learned about ionospheric plasma physics⁷.

Even without the 'heater', the ease with which EISCAT can detect the 'plasma lines' (narrow band echoes at frequencies of approximately $f \pm f_N$, where f is the radar frequency and f_N the local plasma frequency) raises some interesting possibilities. With most other radars, the plasma lines are not easy even to detect. Comparisons have been made between the plasma drift velocities measured by EISCAT and by STARE, a VHF radar that employs coherent scattering from auroral

irregularities. The two radars are complementary in that STARE can obtain extensive measurements over an area of about 400×400 km near Tromsø on a much more frequent basis than can EISCAT, but only in the presence of suitable auroral structures. Data from the two radars agree at low velocities but differ at high velocities, posing interesting questions⁸.

Future plans involve the VHF radar, which will be particularly valuable for studies at great heights and in the middle atmosphere, for which its longer radio wavelength has considerable advantages. Testing of the VHF transmitter is in progress, with the object of obtaining VHF incoherent scatter echoes in the summer of 1985. EISCAT's science is supported by data from the ionosondes, riometers, magnetometers, auroral all-sky cameras, doppler radar and other geophysical instrumentation that is operated in Northern Europe, and of course by cooperation with rocket and satellite experiments. EISCAT will cooperate with the Swedish VIKING satellite this year, and with the International Solar-Terrestrial Programme⁹ from about 1990 onwards.

The EISCAT antennas can also be used 'passively' without the transmitter, for example to record the interplanetary scintillations of quasars, which give information on the velocity and turbulence of the solar wind; and as a long-baseline interferometer, possibly for studying the geodynamics of the Fennoscandian shield. □

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Topography and tectonics

Japanese deep-sea trench survey

from the Shipboard Scientific Staff of the Kaiko Project

LAST summer, the first half of the two-year Franco-Japanese Kaiko programme for exploration of the deep-sea trenches close to Japan was successfully completed with a detailed topographic and geophysical survey by the French research vessel *Jean Charcot*. Now, preparations are underway for this year's programme in which France's new deep-sea submersible SM97, *Nautilus*—launched in October (see *Nature* 31 January, p.339)—will make its maiden scientific dives to explore deep trench features, to collect bottom samples and to install ocean-bottom instruments.

The area under investigation is of particular interest to Earth scientists for it contains the meeting place of the Eurasian, Pacific and Philippine Sea Plates (see the map). To the south, the Philippine Sea Plate makes contact with the Eurasian Plate upon which Japan stands and is subducted beneath it in the 4,500 metre deep Nankai Trough. To the north, the Pacific Plate is subducted beneath the Eurasian Plate forming the deep Japan and Kuril Trenches (deeper than 7,000 metres). At the centre of the map is the classic triple junction where all three plates meet,

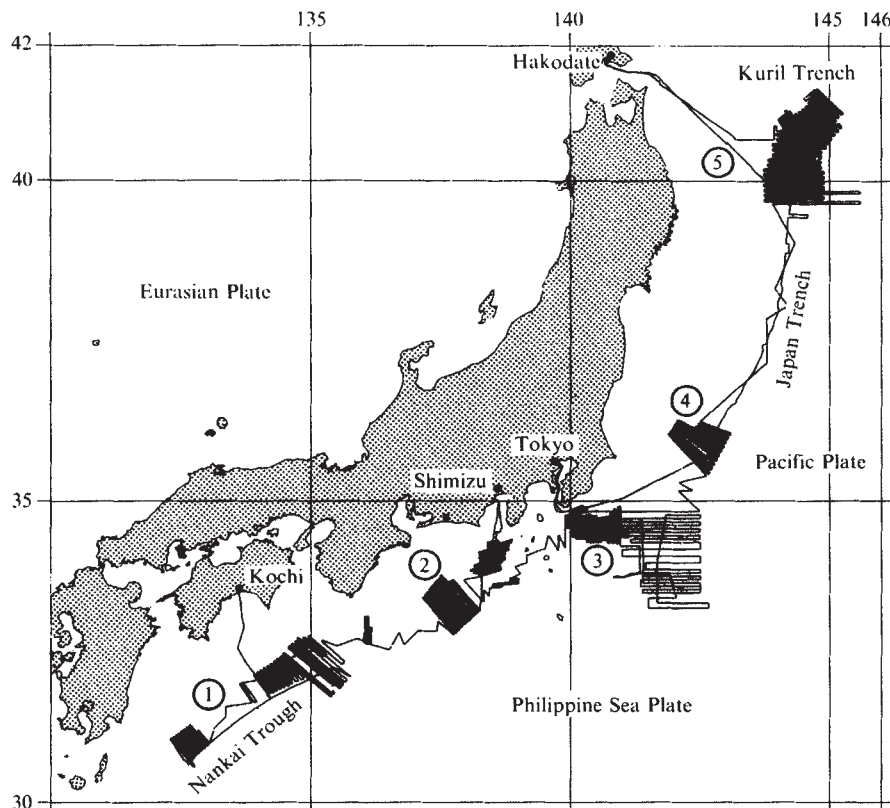
creating the Surugai and Sagami Troughs on each side of the colliding Iwo-Jima island arc. Parts of all these trenches were surveyed along with the remarkable undersea mountain, Dai-ichi Kashima.

The *Jean Charcot* made detailed Seabeam maps of the bottom topography of trench slopes as well as measuring seismic reflection, magnetic total force and gravity, while cruising at 10 knots. The ship's position was fixed with an error of less than 100 metres by the LORAN C navigation system controlled by the Navy Navigation Satellite System and the Global Positioning System.

Four ports of call — Kochi, Shimizu, Tokyo and Hakodate — divided the Kaiko cruise into three legs, each of 18 days. In the Nankai Trough, investigated in leg one, seismic profiling and magnetic mapping revealed the presence of north-south transform faults, roughly perpendicular to the trench axis, in the youngest central part of the basin. These faults are reactivated during subduction, thus controlling the structure of the accretionary prism. The eastern part of the Nankai Trough is complicated by the collision of the Iwo-Jima island arc with Japan. A major basement ridge, the Zenisu Ridge, parallel to the Nankai Trough and 40 km south of it, has been uplifted by 2–3 km. Sediments are piled up and deformed by compression at the foot of the large south-facing basement scarp, suggesting that the ridge is the result of large-scale northward underthrusting and may correspond to a nascent secondary subduction zone.

Faults in the oceanic lithosphere adjacent to the trench axis were also observed on the oceanward slope of the Japan Trench surveyed by legs 2 and 3. They are normal faults dipping both oceanward and landward to form a horst and graben structure, and are either roughly parallel to the trend of the trench axis, as is usual, or at an angle of nearly 60° to it. The latter faults seem to be parallel to magnetic lineaments of the ocean floor, which indicate the strike of the ancient active spreading ridge. Interestingly, no normal faults have been recognized about 100 km east of the trench in the ocean basin near to the crest of the outer swell, indicating that the faults are formed due to bending of the lithosphere as it approaches the trench. Thus it seems likely that faults generated at the mid-oceanic ridge are 'rejuvenated' when the lithosphere is bent near to the trench.

Normal faults were also found in seamounts entering the Japan Trench. The survey clearly showed the Dai-ichi Kashima Seamount, which is about the size of Mount Fuji and is at the southern end of the Japan Trench, is cut into two halves by a large normal fault about 100 km long, as suggested by Mogi and Nishizawa (*Proc. Japan Acad.* B56, 257; 1980). One half is still perched on the rim of the Japan Trench whereas the other half has partially sunk into the trench. Vertical displacement along the fault plane is greatest at the centre of



Map showing the major plate tectonic features investigated by the Kaiko programme and the four ports of call. 1, The Nankai Trench; 2, the Suruga Trench with the Sagami Trench to the east of it; 3, the triple junction, where the Eurasian, Pacific and Philippine Sea Plates meet; 4, Dai-ichi Kashima Seamount; 5, Erimo Seamount, at the junction of the Japan Trench to the south and the Kuril Trench to the north.

the seamount, where it reaches 1,500 m, and decreases towards the ends of the fault. Apparently, young sediments which have partly filled the trench are also vertically offset at the northern extension of the same fault, implying that the fault motion must have occurred quite recently. The Erimo Seamount, situated at the junction of the Japan and Kuril Trenches, is similarly cut by at least three normal faults parallel to the trend of the Kuril Trench.

The Nankai Trough and the Japan Trench were shown to be remarkably different. In the former, plenty of mud and sand is being transported into the trench-bottom axis from the land along several canyons. The bottom of the trough is buried by thick sediments, some of them piled up and accreted to the lower landward slope by the subducting oceanic lithosphere. Mud diapirism was detected within the trough. By contrast, the floor of the Japan Trench is occasionally covered by relatively thin terrigenous sediments which have slumped down from the landward slope, but most of the landslide deposits seem to have been subducted together with the oceanic lithosphere. Along its length the Japan Trench frequently changes direction under the influence of faults that strike obliquely to the trench axis to form a remarkable zigzag. Like the Nankai Trough, thick sediments cover the bottom of the Kuril Trench, at least at the western end which was surveyed. A steep cliff bordering the western margin of the Kuril

Trench seems to have resulted from left-lateral strike-slip faulting, which may record the past strike-slip motion of the eastern Hokkaido relative to the western landmass.

The triple junction of the Izu-Bonin (Ogasawara) Trench and Sagami Trough investigated in leg 2 shows a notable degree of subsidence. A triangular area at the corner of the junction, with an edge of several tens of kilometres, is covered by a huge accumulation of terrigenous sediments. The surrounding ocean floors are all inclined toward the centre of the triangle. These morphological and other geological observations imply that the area is continuing to subside much faster than the rate at which terrigenous matters are transported through the meandering Boso Canyon, which was extensively studied on the expedition and which connects the area to the land. Several deep unfilled holes, where the subsidence rate is particularly high, were also found in the triangular area of the triple junction.

After *Nautilie* has completed this year's dives, planned for June and July, some of the surveyed sites will be nominated for further study by deep drillings and down-hole observation as a part of the Ocean Drilling Program. □

Co-chiefs of the Kaiko project were: Leg 1, Kochi to Shimizu: T. J. Iiyama, University of Tokyo and X. Le Pichon, Université P. and M. Curie, Paris; Leg 2, Shimizu to Tokyo: K. Nakamura, University of Tokyo and V. Renard, IFREMER, Brest; Leg 3, Tokyo to Hakodate: J.-P. Cadet, Université Orleans and K. Kobayashi, University of Tokyo.

Cortical maps

Upside-down world in bat's brain

from Edward Jones

ON PAGE 477 of this issue, M.B. Calford and co-workers report that significant parts of the body-surface map formed in the somatic sensory areas of the cerebral cortex of a flying mammal, the fruit-eating bat *Pteropus poliocephalus*, is reversed in comparison with that in other mammals¹. This correlates with the upside-down posture adopted by the bat at rest (see cover picture) and, Calford *et al.* argue, implies that the cerebral cortex analyses spatial information in terms of the habitual orientation of an animal's body in extra-personal space.

The study is typical of many in which maps of visual space, auditory space or the body surface are being detected in the cortex. The maps in the somatic sensory cortex are revealed by stimulating small portions of the body surface and recording the resulting localized electrical activity in the cortex with microelectrodes. Each map is usually a systematic representation of the body surface, with adjacent parts of the body surface sending information to, and thus represented by, neighbouring groups of cells in the cortical area containing the map² — hence the term 'somatotopic representation'. The somatotopic maps are usually illustrated in popular cartoon form as homunculi, simiusculi, felisculi, rat-tusculi and so on, their numbers limited only by the availability of species and the patience of the investigator, and their names only by the investigator's familiarity with Latin. The maps on page 477 could be called chirunculi or pteropusculi.

In common with all other mammals investigated, this bat has three somatic sensory areas, each with a complete somatotopic map, and multiple visual and auditory areas, each with their own maps. Like other mammals, too, the map of the body surface, though systematic and, thus, somatotopic, is distorted; certain peripheral regions, such as the rhinarium, facial whiskers, the free first digit of the wing and the regions adjacent to it, have disproportionately large representations. *A priori*, therefore, it can be inferred that these regions have a greater density of innervation than other structures, reflecting their functions in this species as the major sensory and manipulating surfaces.

A significant difference between the bat and virtually all other mammals that have been studied^{2,3} is that the mapping of forelimb digits is reversed in two of the maps so that the representations of the tips of the digits point posteriorly instead of anteriorly. The representation of the belly surface of the trunk is also reversed.

These results are interpreted by Calford *et al.* as indicating that the constraints on mapping the body surface in the somatic

sensory cortex include the necessity for maintaining the relative dispositions of body parts to one another and to the head. The bat's forelimb is usually held with the digits directed behind the head during flight and above it during rest; that is, according to the authors, its spatial relationships to the rest of the body are the reverse of those in most other animals. In other words, the relationship between the receptor surface and extra-personal space must be preserved in the cortical map. This has far-reaching implications for the anatomy of the somatic sensory system, because the nerve fibres bearing information to the somatic sensory cortex must have been reshuffled during evolution to create the reorganized map, and their source, the thalamus, must also have been modified.

The analysis of personal and extra-personal space by the mammalian cerebral cortex is an accepted dogma⁴; it is also widely accepted that this is accomplished in terms of a series of two-dimensional maps. It is not too great a flight of fancy to imagine that somewhere in the distant evolutionary past, advantages conferred by this propensity for forming maps constituted an irresistible pressure upon the cerebral cortex, leading it to expand as an enormous flat sheet rather than as a bolus of cells. It is not easy to see what the advantages of map-making are for the cortex, because some degree of spatial

analysis could presumably be carried out by a kind of flying-spot analysis in a bolus of cells that received information from the peripheral sense organs.

Calford *et al.* would argue that the necessity of preserving spatial relationships is a major constraint imposed by the periphery on map-making in the cerebral cortex. That the somatic sensory map is mutable and can be influenced from the periphery is demonstrated in recent experiments on monkeys, where changes in the somatotopic map can be induced extremely rapidly in response to peripheral manipulations, such as severing nerves or amputation of a finger^{5,6}. Whether the spatial domain is pre-eminent seems moot, for the several sub-modalities of somatic sensation must also maintain their differential representations in the same area of cortex. When these are mapped independently, the resulting map has often appeared remarkably unlike that based on somatotopy alone⁷. It is in the co-mapping of space and modality that the somatic sensory cortex is most remarkable. □

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Eukaryotic genes

Are introns structural elements or evolutionary debris?

from Athel Cornish-Bowden

THE discovery of introns — sequences of non-coding DNA that interrupt the coding sequences of genes but are excised from gene transcripts — burst upon an unsuspecting world in 1977. Since then, much information has accumulated about the ubiquity of introns in the genes of higher eukaryotes and their absence from those of prokaryotes and lower eukaryotes, but it is still not really clear why they are there or what they do. There is still argument over whether they were present in the primordial genes and have been eliminated in the interests of efficiency by prokaryotes, or whether, instead, they were introduced after the eukaryotes separated from the prokaryotes. Papers in this issue of *Nature* and the January issue of *Cell* contribute to the debate.

It is scarcely tenable to propose that all introns are functionless, opportunistic

pieces of selfish DNA which have invaded eukaryotic genes by taking advantage of the biochemical machinery that normally excises them. If so, why then did the excision machinery evolve in the first place? At least some introns, therefore, must have had a function, at some stage in the evolution of the gene. The most plausible suggestion is that the existence of introns makes it much easier for large and complex proteins to evolve, as they can be assembled from the small functional units or domains into which introns split genes. Moreover, new functions could in principle be produced by rearranging such domains. Once introns evolved, however, it became possible for parasitic DNA to take advantage of the excision machinery. Thus although some, perhaps most, introns must have a functional origin, it is not necessary to assume this for every intron.

- (A) VKVGVDFGRIGRLVTRAAFNNGKVDIVAINDPFDLHYVMVYFYDSTHGKFGHTVKAEDGKLVIDGKAITIFQE
 † (II) † (III) † (IV)
- (B) LAAALIVMTESGRSAHLVSRYRP RAPIIAVTRNDQTARQAHLRY GVFPLCK
 † (8)
- (C) STCAVFLGGVGLSVIMGCKAAG AARIIGVDINKDKFAKAKEVG ATECVNPD
 † (5) † (6)
- (D) BBBBBBBB aaaaaaaaaaaaaa BBBBBBBB aaaaaaaa [BBBBBB BBBBBB] BBBBBB

Intron arrangement in the mononucleotide binding-site region of pig glyceraldehyde 3-phosphate dehydrogenase (A), chicken pyruvate kinase (B) and horse alcohol dehydrogenase (C), together with the consensus secondary structure (D) in terms of α -helices β -pleated sheets. Amino acids are designated by the one letter code; the positions of introns in the corresponding genes are shown by arrows and numbered according to the authors.

On page 498 of this issue, Schwartz and co-workers suggest that both kinds of intron are recognizable in the glyceraldehyde 3-phosphate dehydrogenase gene of the chicken. They classify the introns as type A, which result from the original assembly of the gene from smaller units, and type B, which have never had any function. Moreover, they believe that type A introns can be further classified according to whether they derive from the creation of a domain from smaller units (A1), from the duplication of a complete domain (A2), or from the joining of two dissimilar domains (A3). Classification of the three introns that occur at or near the boundaries of the four recognized structural domains of the enzyme is straightforward: one is type A2, two are type A3. In addition, the catalytic domain contains a region that makes about 85 per cent of its hydrogen bonds within itself and which also corresponds to an exon — the part of the gene that is translated into protein. Thus, the two introns that flank this exon can be regarded as resulting from

the joining of protein domains and so of type A3.

Apart from one intron in the non-coding region of the mRNA, all of the remaining six introns occur within domains and are more difficult to classify, because both A1 and B type introns would be expected in similar positions. Nonetheless, Schwartz and co-workers believe that sub-domains can be recognized within the domains, and that several of the remaining introns can be interpreted as separating them, so that they are of type A1. Only one intron in the coding region is assigned to type B.

This type of classification can be tested by examining the genes of comparable proteins. Types A2 and A3 introns should occur in other genes at domain boundaries; type A1 introns should occur at homologous positions within homologous domains; but type B introns should occur at arbitrary and unrelated positions. It is of interest, therefore, to examine the hypothesis of Schwartz and co-workers in relation to new work by Lonberg & Gilbert (*Cell* 40, 81; 1985). These workers have determined the structure of the gene for chicken pyruvate kinase and compared it with that of the gene for maize alcohol dehydrogenase (Dennis E.S. *et al.*, *Nucleic Acids Res.* 12, 3983; 1984). All three proteins contain a mononucleotide binding domain with alternating regions of β -pleated sheet and α -helix, though this

domain in glyceraldehyde 3-phosphate dehydrogenase is considerably longer because it contains an extra piece of β -sheet (which does not interfere with the structure of the rest of the domain). The comparison shown in the figure, with arrows indicating the location of introns, is disappointing: intron II of the glyceraldehyde 3-phosphate dehydrogenase gene, classified as type A1 by Schwartz and co-workers, occurs at the boundary between β and α regions, but there is no intron at this boundary in either of the other two genes; intron III, on the other hand, suggested to be of type B, corresponds almost exactly to an intron of the alcohol dehydrogenase gene.

Examining the pyruvate kinase gene as a whole, it is clear that introns are not placed at random in the sequence, and at least two systematic characteristics are evident. The ten exons are of a much more uniform length than one would expect if the coding region were interrupted at random, and the introns tend to fall between discrete regions of secondary structure in the protein. Surprisingly, there is little tendency for introns to occur at domain boundaries: most noticeably, the boundary between domains B and A₂ occurs near the middle of exon 5, the longest exon in the gene. It would be appealing, though entirely speculative, to suggest that an intron at this boundary has been lost, because if it were present it would not only separate the two domains but would also make the exon size even more uniform.

Although there is now much to suggest that introns are an ancient relic of primordial genes, convincing proof must await the discovery of clearly corresponding intron arrangements in genes for that arose by duplication before the separation of prokaryotes and eukaryotes. □

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100 years ago

THE LIFE HISTORY OF THE LYCOPODIACEAE

THE area within which really notable discoveries are possible — at any rate amongst the higher plants — in the field of vegetable morphology is becoming very circumscribed. For some time the complete life-history of the *Lycopodiaceae* has been a missing chapter in our text-books. A paper which will mark its epoch in the history of *Lycopodium* is that for a separate copy of which I am indebted to my friend, Dr. Treub, the accomplished director of the Botanic Garden at Buitenzorg in Java. On his arrival at Buitenzorg, he lost no time in endeavouring to find the prothallia of tropical species. He seems to have all but succeeded in discovering those of *Lycopodium cernuum* in the first year of his residence there. Subsequently, he sowed the spores on the trunks of trees, and after a delay which led him to abandon any hope of success, he obtained satisfactory results from one of the sowings.

In the present paper, Dr. Treub gives an exhaustive account of the prothallium of *Lycopodium cernuum*. It is curious to observe, however, that in artificial cultures he did not succeed in carrying the development further than DeBary had done some time ago with *L. inundatum*. Fortunately, prothallia which he discovered under spontaneous conditions of development exactly fitted in where the others stopped.

W. T. THISELTON DYER

From *Nature* 31, 5 February 1885.

Palaeontology

Back to the trees for *Archaeopteryx* in Bavaria

from Michael E. Howgate

NOBODY expected the latest *Archaeopteryx* conference* to answer all the problems thrown up by this unique intermediate fossil. Indeed some of the more sceptical participants doubted if any of the problems would be nearer resolution after the event. However, a consensus of sorts emerged; several theories met their Waterloo; agreement was reached on some outstanding problems; and other contentious areas were set for resolution in the foreseeable future.

* 'The International *Archaeopteryx* Conference', Eichstätt, FRG, 11-15 September 1984. The conference proceedings will be available through Dr. G. Viohl, Jura Museum, Willibaldsburg, 8078 Eichstätt, FRG.

To help the proceedings, three of the six specimens of *Archaeopteryx* were present: the counterpart of the isolated feather imprint; the incomplete Tyler specimen; and the perfectly preserved (apart from the feather impressions) Eichstätt specimen accompanied by a specimen of the small dinosaur *Compsognathus* from the same Upper Jurassic formation. The Berlin specimen of *Archaeopteryx* was unable to attend, as it is on loan to Japan; the British Museum (Natural History) specimen was in too fragile a condition to travel, but an excellent video and slides were sent instead;

and, most disappointing for the organizers, the local private owner of the Maxberg specimen refused to allow any palaeontologists to view it, a position which he has maintained for the past ten years. This prompted the conference to send a letter to the Bavarian State Government urging them to ensure that objects of such great importance should be made available to the scientific community.

The two debates that dominated the proceedings concerned the ancestry of *Archaeopteryx*, interpreted as 'nearest sister-group relationship' by the cladists, and its functional anatomy—was it an arboreal flyer or a terrestrial glider? One of the three competing hypotheses of ancestry had fallen by the wayside even before the conference started: Alick Walker (University of Newcastle-upon-Tyne), originator of the view that *Archaeopteryx* is more nearly related to crocodilomorphs than to either theropod dinosaurs or 'primitive' thecodontians, has changed his opinion. He now regards the common specializations of the auditory region and jaw articulation seen in birds and crocodiles as the result of parallel evolution from a thecodontian common ancestor. This left Larry Martin (University of Kansas) maintaining the crocodilomorph relationship on the basis of tooth shape, a character this author regarded as 'agnostic' to the debate, as the teeth of *Archaeopteryx* and later Cretaceous toothed birds could be equally well be derived from an early crocodilomorph, a theropod dinosaur or pseudosuchian (or even proterosuchian) thecodont.

In tackling the remaining hypotheses, Jaques Gautier (San Francisco) marshalled a host of synapomorphic characters which indicated that *Archaeopteryx* was morphologically a dinosaur. The Eichstätt

skeleton (see figure) was indeed so like a small dinosaur that for 20 years from the time of its discovery in 1952 it was referred to the small coelurosaurian species, *Compsognathus*. Proponents of the alternative hypothesis, that *Archaeopteryx* and birds only shared a more 'primitive' thecodontian ancestor with the dinosaurs, were left with the task of explaining the large amount of parallel evolution that must have taken place and for which there is no fossil evidence. The counter-attack launched by J.R. Hinchcliffe (University of Wales, Aberystwyth) focused on the critical question of the relationship between the digits of the wing of modern birds and those of *Archaeopteryx*. Hinchcliffe presented embryological evidence that the three digits in a bird's wing are the middle trio (2:3:4) of the ancestral five. Palaeontological evidence, however, favours a digital formula of 1:2:3 in the manus of theropod dinosaurs, although the evidence is inconclusive because only four digits are known even in the most primitive specimen. M. Hecht and S. Tarsitano (City University, NY) believe that both lines of evidence are correct and that therefore the digital-reduction pattern was different in dinosaurs and birds, consequently they could only be related at the thecodontian level. J. Ostrom (Yale University) and Gautier felt that the embryological evidence is wrongly interpreted and that the digital-reduction pattern is 1:2:3 in both birds and dinosaurs. Lastly, R. Thulborn (University of Queensland) argued that the digital-reduction pattern in theropod dinosaurs is wrongly interpreted; it is his opinion that 2:3:4 is the pattern for both birds and dinosaurs.

Although the consensus was in favour of a theropod ancestor for *Archaeopteryx*, the

possibility that one key synapomorphy, if falsified, would reduce all the other synapomorphies to the status of parallelism and hence irrelevant to the debate, should be a lesson to the more dogmatic cladists.

The question of whether *Archaeopteryx* was arboreal or terrestrial was answered by Derek Yalden (University of Manchester), who proved to the satisfaction of everyone that the claws on the wings were adapted for climbing squirrel-like up tree-trunks. The claws, which are narrow, highly curved and fine-tipped, are distinct from raptorial claws but resemble those of trunk-climbing birds and mammals. Also, in contrast to current reconstructions, the claws did not protrude in front of the wing on free digits but must have pointed ventrally to allow digits 2 and 3 to support the primary feathers. Siegfried Rietschel (Karlsruhe) suggested that the claws also had a preening function and that the enigmatic impressions of feathers on the Berlin specimen are, in fact, ghost impressions of primaries which had been twisted under neighbouring feathers. Thus *Archaeopteryx* had a fully modern wing. Despite that, it could not have been a good flier as it lacked a supracoracoideus pulley-system to act as a wing elevator and possessed a ventro-laterally directed glenoid which prohibited the wing from being elevated much above the horizontal (Tarsitano), thus effectively limiting its wingbeat to half a stroke.

While *Archaeopteryx* was, by consensus, allowed back up into the trees, its putative ancestor, the 'pro-avis', was still the subject of much debate. R.P. Balda, G. Caple and W.R. Willis (Northern Arizona University, Flagstaff) consider it unlikely that a gliding animal could give rise to an active flier at realizable speeds, as the recovery stroke of the wing beat would cause a significant loss of power and height, such that it would tend to shorten rather than lengthen the glide path. Consequently they envisage the proto-bird as initially using its proto-wings for balance while running, jumping and turning until able, possibly downhill, to take-off at high speed. But as J.A.V. Rayner (University of Bristol) pointed out, there are no mechanical barriers in the shift from gliding to active flight, as long as the glide speed is sufficient. Moreover, the calculated maximum speed of *Archaeopteryx*-sized coelurosaurian dinosaurs is only a third of the necessary speed to attain take-off, ruling out even a partially gravity-assisted downhill take-off (Thulborn).

As a final fillip to the conference, Ostrom revealed the earliest known drawing of the Berlin specimen, showing the presence of contour feathers along the back and legs and what appears to be a tuft of feathers behind the skull, all long since prepared away. The drawing will be published as the frontispiece to the conference proceedings. □

IMAGE
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The Eichstätt specimen of *Archaeopteryx*, which was considered to be a dinosaur of the genus *Compsognathus* until the faint feather impressions were noticed. (Photo: Dieter Kriebel.)

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CG doublet difficulties in vertebrate DNA

SIR — Contrary to the idea proposed by Adams and Eason¹, the dinucleotide CG does not appear to be less avoided at higher G + C contents in vertebrate DNA sequences. The most meaningful relation in terms of the methylcytosine deamination hypothesis for the suppression of CpG¹⁻³ is the difference between the normalized frequencies of doublets CG and TG as a function of % G + C. These normal statistics refer to the doublet frequencies expected from base composition and are calculated from permutations of the bases in the sequence⁴. I have therefore made plots of CpG suppression (CG - TG) versus % G + C for all human, mouse and chicken sequences of suitable length in our information system ACNUC, which includes the latest GenBank release⁴. No correlation between the two parameters is found. The curves of CG - TG against % G + C are flat with high dispersion for each species. A few sequences do show less avoidance of CpG over 60% G + C, but this is not a general trend. Indeed, although the CG - TG dispersion is large at all G + C contents with the 84 human sequences studied, it is greatest above 60% G + C.

Another test of the idea was made by sectioning the Epstein-Barr virus genome sequence (in latent form it can be highly methylated). The 172 kilobase sequence was divided into 17 segments of 10,000 bases each and the normal statistic CG - TG plotted against % G + C (since the statistic is cumulative the best comparison is with sequences of exactly the same length). This likewise gave a flat, dispersed curve. Furthermore, *Drosophila* sequences, which are not methylated^{3,5}, also produced a curve of like appearance. Finally, plots for *Escherichia coli* and phages T7 and lambda are rather similar to those above (the T7 and lambda complete genome sequences were cut into 2,000-base segments for the analysis).

To add to Max's findings², in my human workfile seven sequences do show somewhat less CpG avoidance than the others; *c-myc* 5' end; enkephalin 5' flank and start of intron C; alpha-actin mRNA; *c-myc* exon 2; enkephalin in exons 1 and 2; tRNA Glu gene and flanks; 45S rRNA transcription origin region (which at 75.3% G + C shows no evidence of CpG suppression). The last five sequences have more than 60% G + C, but nine others of over this amount reveal strong CpG avoidance. In the mouse sample (100 sequences of 1-2 kilobases each, 27 from segmenting longer sequences), the seven of lowest CpG avoidance were: hypoxanthine phosphoribosyl transferase exon 1; 5' end 45S rRNA precursor; first 1,240 bases of MHC class II H2-IA-beta exons 2-6; RSRGO repeat element 5' to rRNA transcription origin; first 1,180 bases of MUSCFOS; second half of MUSCMYCONC; 5' flank, exons 1 and 2 and first introns in MHC class I gene

Q10. The first two sequences have over 60% G + C. Thus instead of a general increase in CpG normalized frequency at high G + C contents, we see particular sequence families exhibiting low CpG avoidance, while taken altogether sequences reveal the avoidance at least as strongly above 60% G + C as below. I wish I had a new hypothesis for the rarity of CpG in DNA.

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SIR — In a recent *News and Views* article¹, I discussed our published observations on "CpG-rich" segments of mammalian genes, and described a model we proposed² that might explain local persistence of this usually rare dinucleotide in 5' regions of certain genes. We suggested that these regions might have been protected from the usual mechanism of CpG loss (deamination of 5-methylCpG to TpG) if these CpG-rich regions were undermethylated in germ-line DNA. The article provoked several responses, including some published in *Nature*^{3,4}.

Erickson³ addresses the methylation status of class I MHC genes, a category of genes in which we noticed that CpG-rich regions occur consistently in the region 5' of intron 3. Erickson cites his experimental evidence that class I MHC genes of the mouse are highly methylated in sperm DNA, which may seem to contradict our undermethylation model; but I believe that his data do not provide a good test of this model. The particular experiment referred to (Fig.3 in ref.5) shows a Southern blot of mouse sperm DNA digested with *MspI* and *HpaII* and probed with a mouse class I MHC probe. If the CpG-rich 5' regions of the MHC genes were completely demethylated while the 3' regions were highly methylated, the 5' regions might be so thoroughly digested at closely spaced *HpaII* sites that the fragments generated from 5' regions of the genes would be too small to be detected in routine Southern blots. (In the H-2L^d sequence, for example, the largest *HpaII* fragment contained in the CpG-rich region 5' of intron 3 is 197 bp; most fragments are less than 100 bp.) Therefore, one might reasonably predict that the only fragments detectable on routine Southern blots of the *HpaII* digest would be large fragments generated from the highly methylated 3' end of the gene, yielding bands similar to what is observed in Erickson's Fig.3. Since the undermethylation model is consistent with the Southern blot reported in Erickson's paper, while this blot is also consistent with the opposite prediction (methylation of the 5' regions), it is apparent that this experiment does not test the

model. (This does not imply a defect in Erickson's data; his experiments were simply designed for other reasons than to explore this particular model.) An additional caveat should be mentioned. Mature sperm represents a state of male germ-line DNA that is terminal and relatively transient compared with that of the long-lived precursor stem cells, cells which may have a different pattern of DNA methylation than mature sperm. Therefore the methylation status of mature sperm DNA may not reflect the methylation pattern most significant in influencing the accumulation of deamination mutations over successive generations. This caveat obviously applies equally well to the experiments that seem to support our undermethylation model by demonstrating regional undermethylation in CpG-rich regions of several genes in mature sperm (as cited in the *News and Views* piece).

Adams and Eason⁴ suggest that G + C-rich regions of mammalian DNA may be held so tightly as a double helix that deamination of 5-methyl-cytosine residues, a reaction thought to occur only on single-stranded DNA, may be prevented in these regions; as a consequence, deamination-caused 'CpG suppression' would be absent in these G + C-rich areas. This provides an alternative model to the one we proposed explaining CpG-rich regions as a consequence of undermethylation of these regions in germ-line DNA.

One theoretical reservation that I have about the 'tight helix' model is that the notion that deamination occurs only on single-stranded DNA is based on *in vitro* experiments on deamination events catalysed by bisulphite^{6,7}. Since the requirement of single-stranded DNA for the bisulphite-catalysed reaction may result from poor steric access of the catalyst in double-stranded DNA, it is not necessarily true that the *in vivo* deamination, not bisulphite-catalysed, would show the same preference for single-stranded DNA.

If one accepts the extrapolation and assumes that *in vivo* deamination also requires single-stranded DNA, then the tight helix model seems to apply well to the genes we considered in our earlier paper², since in these genes the regions that are relatively CpG-rich by and large are C + C-rich also. However, this correlation is not always found. Mouse satellite DNA is composed of a highly repeated DNA segment which demonstrates no CpG suppression. In the 234-bp unit sequence⁸, the observed CpG frequency (3.4%) is almost exactly that predicted from G + C content, yet this sequence does not fall into the category of G + C rich sequences described in the tight helix model since the G + C content is only 36.8%. It does, however, fit the undermethylation model since available data^{9,10} suggest that this satellite DNA is undermethylated in germ-line cells (mature sperm and spermatogonia) although it is highly methylated in other tissues. Furthermore, even when CpG

when CpG suppression is absent in a G + C-rich region in accordance with the tight helix model, the germ-line undermethylation model is not excluded. Thus in bovine satellite I DNA, where CpG suppression is essentially absent and G + C is high (about 57%), genomic sequencing experiments have indicated that these sequences are undermethylated in sperm but not in somatic (thymus) DNA (J. Herbert Taylor, personal communication).

Therefore I believe that, while the tight helix model offers an attractive alternative that may explain some CpG-rich regions, available data do not warrant abandonment of the undermethylation model. To test the undermethylation model, we are planning experiments that may reveal the methylation status of some CpG-rich regions of MHC genes in sperm DNA; we do not presently endorse any particular prediction for the results of these experiments. Even if further data should favour the tight helix model as a mechanistic explanation for the maintenance of CpG-richness, the conservation of this feature in several MHC genes across three species suggests some functional significance (of either CpG-richness or G + C-richness) that remains to be explained.

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In search of physical reality

SIR — T.W. Marshall's review¹ of my book *In Search of Reality* calls for some serious factual qualifications.

The question at issue concerns (in Marshall's words) the possibility or impossibility of physical theories of a "local realist type" essentially built along the lines of Einstein's physical realism (as is well known, Einstein's locality requirements were an essential part of his criticism of the Copenhagen philosophy). Marshall expresses the view — presented by him as certain — that *even within the domain of such theories* there is "no difficulty at all in explaining the data obtained in the experiments so far performed", provided only that determinism is not imposed and that the domain of the theories in question is not arbitrarily restricted to models picked up within too narrow a range.

This claim is highly misleading in that it

ignores the main result of the Bell theorem, namely, that Einstein's local realism is incompatible with some elementary and in principle verifiable predictions of quantum mechanics, quite independently both of models (deterministic or indeterministic) and of any actual experimental result (proofs such as the one transcribed in plain words in the book are, despite superficial appearances, fully general in this respect). Marshall's claim is even plainly erroneous since, when they turn their attention to the results of the experiments so far performed in this field (by Clauser, Fry, Aspect and others), realist physicists (including those who sympathize most with the Einsteinian ideals) generally stress that conceptually it is, by now, if not logically impossible, at least extremely difficult to preserve Einstein's local realism; for, they say, it seems hopeless to do so without attributing the agreement found between the experimental data and the quantum predictions to some coincidence; and this, on the other hand, seems quite unbelievable in view of the quality of the agreement in question. In comparisons of this kind, the inefficiency of the counters is extremely important, but, contrary to a suggestion by Marshall, it does not by itself remove the difficulty at issue — see, for example, refs 2 and 3 for detailed quantitative data and evaluations.

Here and there, various more-or-less convincing attempts are now being made to build up realistic *nonlocal* theories. But it can safely be asserted that a plain regression to the kind of local physical realism that Einstein so strongly advocated is ruled out.

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T.W. MARSHALL REPLIES — Bernard d'Espagnat says it is "extremely difficult if not logically impossible" to construct a local realist explanation for the results of photon-correlation experiments of the Aspect type, while I, in my review of his book *In Search of Reality*, claimed that it is "not at all difficult". I have reached what may seem to be the opposite conclusion from d'Espagnat as a result of reading precisely the literature he cites above in support of his view.

How is that possible?

First we should dispose of the suggestion of "logical impossibility". What d'Espagnat is saying here is that Bell's theorem demonstrates that an ideal experiment, with perfect detectors, will give results either corroborating the quantum theory of measurement and refuting all local realist theories or the opposite. In the absence of such an ideal experiment,

the assessment of real experiments, such as those of Aspect, becomes one of distinguishing between "extremely difficult" and "not at all difficult", which shows that d'Espagnat and I have made opposite value judgements.

The review article of Clauser and Shimony¹ makes it quite clear what is at stake here. We can discriminate between the quantum model and "the family of local realist models" with an experiment using low-efficiency detectors, but only if we restrict the latter by imposing an additional restriction called "no enhancement". From the Aspect experiment, we might claim that local realist models satisfying the no-enhancement restriction are no longer possible. (Even this claim is, however, over-ambitious, because the procedure by which a rather weak "signal" is extracted from a rather large "noise" background is open to criticism².)

What is this no-enhancement restriction? Essentially it imposes on all conceivable local realist theories certain features of the quantum model: that light is transmitted as photons and that the successive interactions of a photon with first a polarizing device and then a photomultiplier are statistically independent. By dropping these assumptions, my colleagues Franco Selleri, Emilio Santos and I have been able, without difficulty, to construct several local realist models³⁻⁵, giving coincidence counts which are consistent with Aspect's results⁶. The imposition of the no-enhancement restriction begs the question in favour of the quantum model, much as the von Neumann "theorem" did at an earlier stage in the debate.

If anyone suspects that this is an "angels on a pinhead" type of controversy, it should be noted that our criticism of the data analysis in existing experiments has shown us the correct way to analyse the coincidence data from low-efficiency detectors⁷. This research indicates that a discrimination between the quantum model and a much wider family of local realist models, not subject to the no-enhancement restriction, is possible.

In the meantime I think that most scientists, given a choice between abandoning physical reality and abandoning such an *ad hoc* hypothesis as "no enhancement", will unhesitatingly choose the latter course. This must mean conceding that Einstein's criticism of the Copenhagen philosophy remains valid — for some of us, overwhelmingly so.

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Global and seasonal variability of the temperature and composition of the middle atmosphere

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The Stratospheric and Mesospheric Sounder on board the experimental meteorological satellite Nimbus 7, launched in 1978, was designed to study the global structure, radiative properties, chemistry and dynamics of the stratosphere and mesosphere. The instrument obtained data over a four and a half year period, until spring 1984. This review considers the results obtained and looks forward to an advanced version now under construction.

IR RADIOMETRY from Earth satellites is now a standard method for measuring atmospheric temperature profiles, both for experimental and operational purposes¹. As such remote sounding approaches maturity as a tool for, for example, weather forecasting, research groups are trying to develop new types of sensors which will increase the scientific applications and achieve higher accuracy and precision. The NASA experimental meteorological satellite Nimbus 7 was launched in October 1978; it contained the Stratospheric and Mesospheric Sounder (SAMS) which combined two novel techniques, limb scanning² and pressure modulator radiometry³, that had been first used on Nimbus 6 in separate instruments.

After exceeding its design lifetime by more than 3 years, the SAMS recently developed a malfunction in its scanning optics and has stopped taking useful data. The large amount of information which has been collected means that we can assess what such an instrument can achieve. This is particularly appropriate, because a new version, the Improved Stratospheric and Mesospheric Sounder (ISAMS), is being designed currently. This will be launched in 1989 on the NASA Upper Atmosphere Research Satellite.

Objectives and methods

The SAMS instrument has been described in detail elsewhere⁴. The limb scanning technique involves viewing the atmosphere at a tangent to the surface, rather than downwards as performed previously, to obtain higher vertical resolution². A pressure modulator radiometer (PMR) is a correlation spectroradiometer which uses a sample of the atmospheric gas under investigation (usually carbon dioxide for temperature sounding) as a spectral filter to obtain very high effective spectral resolution³. Together, these techniques offer the possibility of making quantitative measurements of IR thermal emission from the spectral bands of gases which are present in the middle atmosphere in small amounts, without the need to use cooled detectors and optics. These were difficult to accommodate in spacecraft at the time SAMS was designed without seriously limiting the lifetime of the investigation.

SAMS was designed to measure the gases CO₂, H₂O, CO, CH₄, NO and N₂O whose radiances depend on both abundance and temperature. Because CO₂ is mixed uniformly over the altitude range of interest (10–90 km) and its abundance is quite accurately known, CO₂ radiances are used to retrieve temperature structure. These temperatures may then be used to retrieve abundances for the other five species. Retrieval methods for temperature and composition are discussed in detail elsewhere¹.

NO and N₂O were measured because of their importance for the cycles of ozone photochemistry in the stratosphere. N₂O is the main source of chemically-active nitrogen in the atmosphere,

originating at the surface of the Earth in amounts which have been enhanced recently, for example by the increased use of artificial fertilizers. The main reactive products of N₂O dissociation in the stratosphere are NO and NO₂; observations of the latter were not attempted by SAMS because of difficulties with retaining such a reactive gas in a modulator cell (although a special modulator is being developed for this application in ISAMS). NO₂ was measured by the LIMS (Limb Infrared Monitor of the Stratosphere) radiometer⁵, also on Nimbus 7, which measured the distribution of HNO₃ as well as NO₂ and thus obtained a data set which complemented that from SAMS. LIMS was more conventional than SAMS in that it employed multilayer interference filters for spectral selection, but included the use of a reservoir of cryogen (solid CH₄ and solid NH₃) to cool the detectors and associated optics for greater sensitivity. This factor, however, limited the operational lifetime to seven months.

Nimbus 7 was launched on 28 October 1978 into a 100° inclination Sun-synchronous orbit at an altitude of ~1,000 km, from which SAMS limb-scanning measurements cover the globe from ~50° S to 70° N with measurements repeated at any given location twice per day, always at the same two local times. The SAMS field of view, projected on to the limb of the Earth, was ~8 km high and 100 km wide, the former determining the vertical resolution of the measurements. Vertical coverage was obtained by scanning the field-of-view up and down the limb; the repetition rate of the scan was variable and so, therefore, was the horizontal sampling rate. The latter was typically 2°–4° of latitude, which can be compared with the separation of successive orbital tracks of ~25° of longitude.

The measurements by SAMS of H₂O, CH₄ and CO were intended, in addition to revealing the global distributions and seasonal variabilities of these species, to shed light on the problem of the middle atmosphere water budget⁶. Water enters the stratosphere through the tropopause in ways which are not understood fully and is destroyed above the mesopause by photodissociation. At intermediate levels it is produced photochemically by the oxidation of methane through a series of reactions which involve CO as an intermediate product. CO is produced at high levels by photodissociation of CO₂ as well as by combustion processes on or near the Earth's surface. Extensive measurements in space and time are essential for unravelling the various contributors to a complex balance in which radiation and dynamics are also important.

Results

The first step when using data acquired by new techniques is to validate the measurements and the algorithms employed to convert raw IR radiances into useful geophysical quantities. In the case of SAMS, the latter involved removal of or accounting

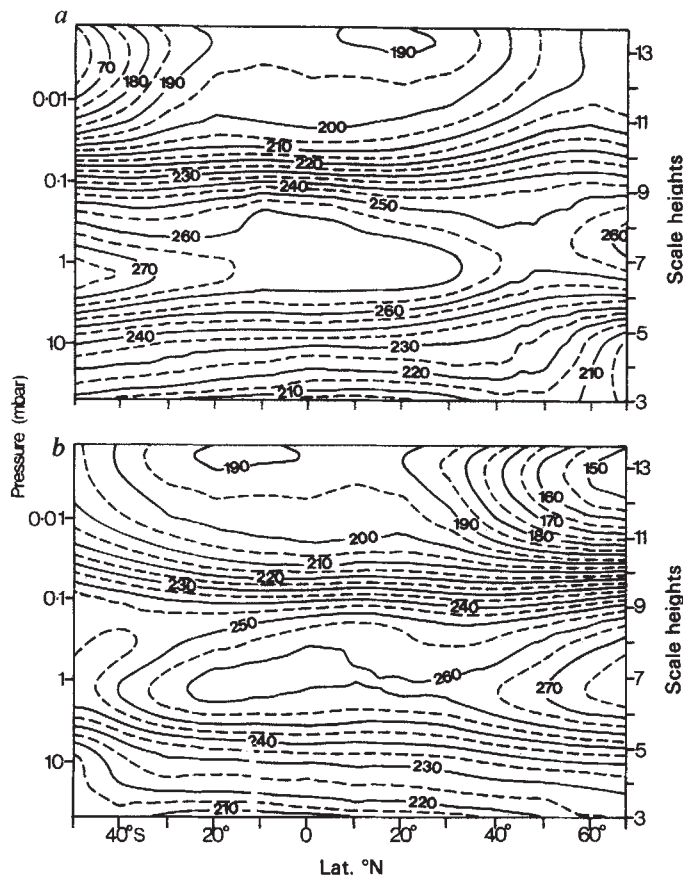


Fig. 1 Zonal mean temperature fields from the SAMS for: *a*, 15 January 1979 ('typical' winter); and *b*, 16 July 1979 ('typical' summer). See text for discussion of the vertical scales.

for the effects of instrumental temperature fluctuations induced by orbital fluctuations in solar and terrestrial heating and by duty cycling of power to the instrument. It is necessary also to determine exactly where in the atmosphere the instrument is pointing from the data itself, as spacecraft roll uncertainties can be 1° ; these correspond to tangent height uncertainties of ± 25 km at the limb. The algorithms developed for this purpose⁷ can retrieve the mean attitude of an instantaneous field of view to better than $\pm 0.002^\circ$, or about 100 m along the vertical direction at the limb. Detailed comparisons which have been carried out⁸ between SAMS temperature retrievals and simultaneous measurements by rockets and radiosondes suggest that discrepancies in the temperatures of 1–2 K and in the retrieved attitude of up to $\pm 0.006^\circ$ (360 m) may still exist.

Temperature structure

Figure 1 shows examples of temperature fields retrieved from SAMS data for typical days about six months apart. The temperature measurements are obtained as a function of pressure because the instrument has no knowledge of height above the surface. A 'pseudoheight' scale can be generated by taking the logarithm of pressure (right-hand scale in scale height units; mean scale height is ~ 7 km). The first-order symmetry of the atmosphere with season, as well as the major seasonally-developing features such as the warm summer stratosphere and the cold summer mesopause region can be seen in Fig. 1. The use of very large data sets of the type from which these examples were taken for studying atmospheric dynamics (circulation and waves), stratospheric and mesospheric warmings, seasonal variations and climatology and budgets of heat, energy and momentum has been described elsewhere^{1,2}.

Figure 2 shows SAMS data during a period of planetary wave activity in January 1981. The geopotential height field was derived from the temperature field using the hydrostatic relation

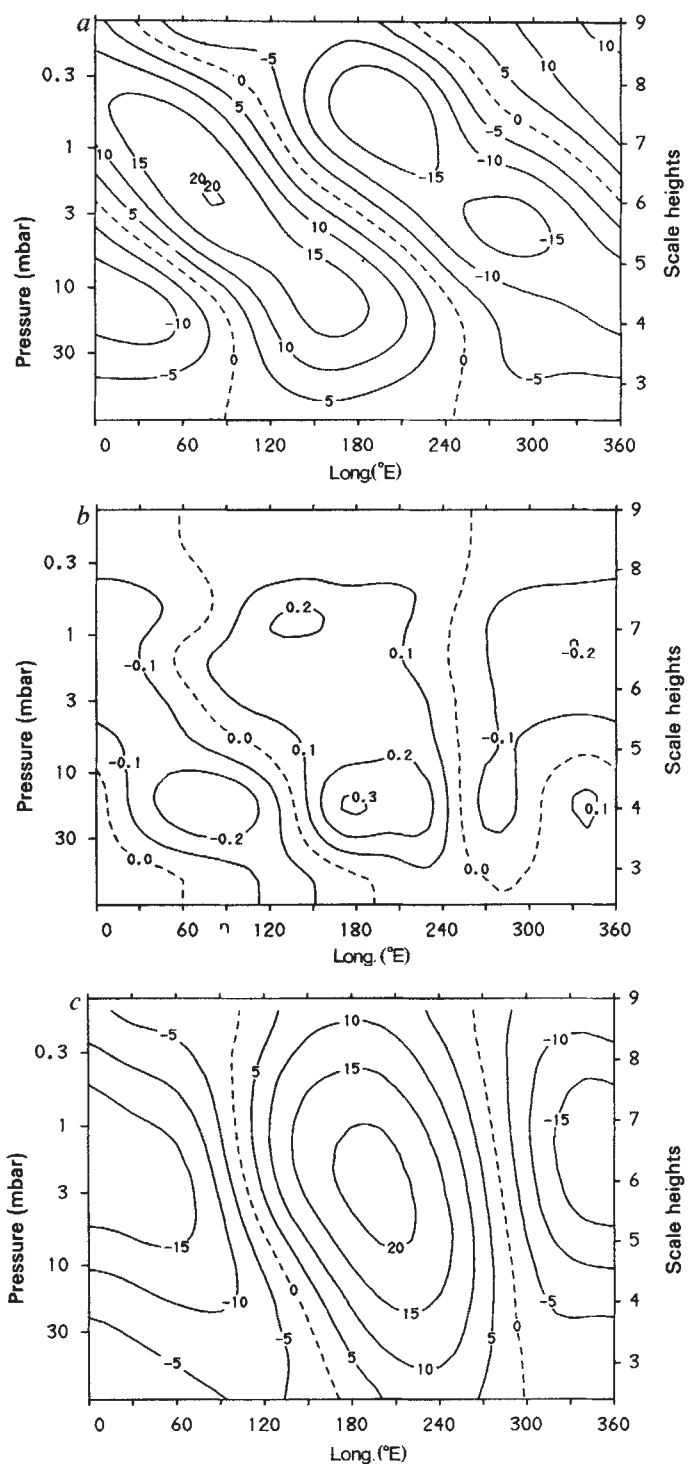


Fig. 2 Longitude-pressure sections of deviation from the zonal mean for: *a*, temperature in K; *b*, methane in parts per million by volume (p.p.m.v.), *c*, geopotential height in hundreds of metres, all on 23 January 1981 (after ref. 22).

and the NMC 100-mbar geopotential height analysis. The westward tilt of the temperature and height perturbations with height is well understood and an almost invariant feature of stationary planetary waves, but Fig. 2 shows that the field of methane shows the same behaviour. There is a marked positive correlation between perturbations of methane mixing ratio and geopotential. For a steady state with a westerly mean flow and for the normal vertical and latitudinal gradients of mixing ratio, such a correlation is expected for small amplitude waves. Conditions at this time, however, were not steady (there was a strong

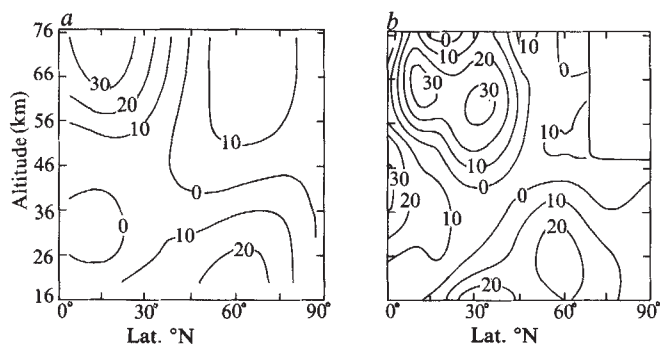


Fig. 3 Zonal winds for 1 February 1981: *a*, computed and *b*, measured, as a function of latitude and height. The empty rectangle in *b* represents a region of no satellite data from the SAMS, because of the limb-viewing geometry; below 1 mbar pressure the data from the Stratospheric Sounding Unit on Tiros-N have been employed. After ref. 10.

cross-polar wind flow) so this good correlation was not expected; it is the subject of further study.

An important use of temperature and geopotential height fields is in conjunction with numerical simulation models, both to provide valid initialization data and to gauge the model's performance. This has been done¹⁰ using a time-dependent quasi-geostrophic model of the Northern Hemisphere featuring spherical geometry with dissipation and a zonal mean and eddy (1 zonal wave) component, similar to that used previously¹¹. The model domain is 2.8–14.6 pressure scale heights (~20–100 km) at intervals of 0.2 and 0°–90°N at intervals of 5°. The time step was 2 h. The minor warming of early February 1981 was simulated, beginning on 23 January with the observed zonal mean state and zero eddy state, using observed forcing at the bottom boundary, except that the amplitude was modified to start at zero at the beginning of the run and allowed to build up. The agreement between the model and measurements was very satisfactory and a change of the observed zonal mean winds from westerlies to easterlies after the warming was successfully simulated in magnitude, timing and location. Figure 3 shows the agreement found for 1 February. The run was repeated using an idealized initial wind field, typical of winter, but not the actual conditions prevailing at the time. The same lower boundary forcing was used, but no warming resulted (the zonal winds changed very little and stayed strongly westerly), confirming the importance of the atmospheric conditions before a warming, not just the bottom boundary forcing.

Volcanic dust

Although SAMS was not designed to measure volcanic emissions, it has been possible to observe at some wavelengths the cloud from the eruption of the Mexican volcano El Chichón, which occurred during March and April 1982. Scattering of solar radiation in the 'B1' channel at 2.7 μm shows the cloud most clearly, but this observation is restricted to the dayside of the orbit. The B1 channel was designed to measure water vapour at around 90 km by observing solar radiation scattered by resonance fluorescence. No significant fluorescence signal was expected in the lower stratosphere. Thermal emission at 8 μm in the 'C2' and 'C3' channels gives day and night coverage, but the signal-to-noise level is poorer, and the emission from the volcanic aerosol is combined with that from CH_4 and N_2O , as it was these that the channels were designed to measure.

The El Chichón data have not yet been analysed in detail, but they should enable us to follow the evolution of the cloud in three dimensions (although at a vertical resolution of only 8 km) and provide an opportunity for studying the transport by the atmosphere of this point injection of material.

Some preliminary results reveal that the opacity maximum settled initially at ~25 km (Fig. 4), and spread out mainly towards the west, completely circling the globe in about 20 days.

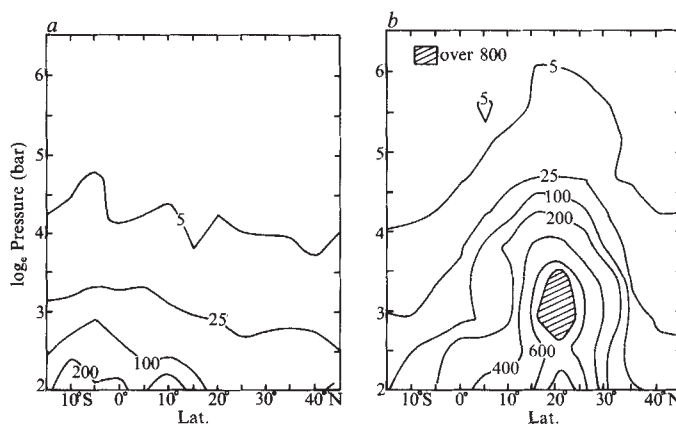


Fig. 4 Wide-band emission near 2.7 μm in telemetry units from the SAMS: *a*, before; and *b*, a month after the El Chichón eruption, indicating the spread in height and latitude of the dust cloud.

The cloud remained between Equator and 35° N until autumn 1982. By February 1983 the level of peak opacity was below 15 km and polewards of 50° N.

Minor constituent abundances

The SAMS NO measurements were of poor quality because of a chemical and a mechanical problem in the NO modulator. The CO , CO_2 4.3- μm band and water vapour 2.7- μm band data have poorer signal-to-noise ratios than was intended because the detector cooling for these short-wavelength channels (by a passive radiator) was less than expected (about 170 K was realized, rather than 160 K planned). All of these channels provided useful scientific data, however, some of which has been analysed already. The long-wave CO_2 and water vapour channels (at 15 μm , and covering 20–100 μm , respectively), the CH_4 and N_2O channels, all functioned essentially as expected over the lifetime of the instrument.

All of these measurements require accurate spectroscopy to retrieve the constituent abundances with sufficient precision that global and seasonal variations can be assessed. Validation by comparison with observations by other techniques is important for temperature measurements. In making intercomparisons, the time and place of the measurements must be the same or at least correspond to similar latitudes and times of year. The latter is more feasible, but there may be misleading conclusions due to short-term variability and spatial inhomogeneities in the composition field. Remote sensing and *in situ* measurements of various categories never sample the same volume of air and each has errors. The way forward is to obtain the largest possible data set by as many different techniques as can be devised and by emphasizing the usefulness of simultaneous and coincident data sets. This is being achieved by multiple sensors on satellites and by coordinated balloon launches incorporating several different approaches to the measurement of the same quantity. Ground-based remote-sensing measurements have an important role by measuring fluctuations with time at a single location.

Once the measurements are understood reasonably well, their interpretation can begin; this is complex because most species have several sources and sinks distributed through the atmosphere. CO , for example, is produced: (1) at the surface by combustion and propagated upwards; (2) in the upper atmosphere by photodissociation of CO_2 and propagated downwards; and (3) in the middle atmosphere by the incomplete oxidation of CH_4 . Water vapour is removed high in the atmosphere by dissociation and lower down by condensation. The major difficulty in understanding the physics and establishing the contributions of each process under various conditions is the interdependence of radiative, chemical and dynamical processes. For example, if water vapour is observed to increase in the stratosphere with time of day, this could be due to increased

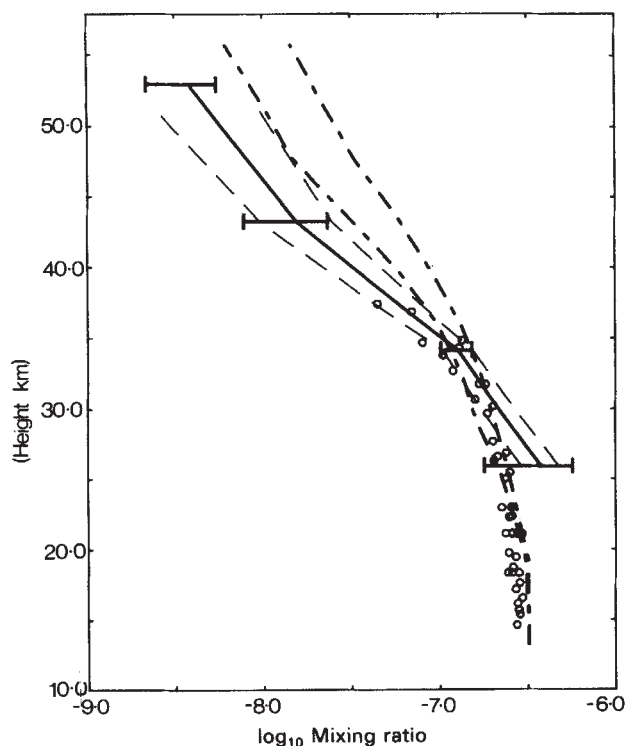


Fig. 5 Comparison of the SAMS N_2O 1979 annual mean profile for 10°S – 10°N (solid line) with other measurements ($\circ$, WMO 1982). Dashed line shows the standard deviation of the monthly mean profiles for that latitude band and the horizontal bars are the estimated accuracy. The heavy dotted-dashed line shows the annual variation observed in the Oxford two-dimensional photochemical and dynamical model over the same latitude band.

production or to advection of moist air into the region from elsewhere.

Progress is possible by using models of the atmosphere in which all of the important processes are represented. The observations can then be compared with the model's predictions and experiments to investigate the sensitivity of the measurable quantities to the processes. Even with modern computers, no model can include all the physics at a resolution comparable with the scales of atmospheric variability, so different models are characterized by their approaches to parameterizing radiative transfer, photochemistry and fluid dynamics in ways which approximate the true behaviour of the atmosphere usefully while remaining economical. Apart from these practical considerations, many aspects of atmospheric behaviour are not well understood, so that introducing appropriate parameterizations is a major challenge.

Figure 5 shows an example of a SAMS N_2O profile compared with a selection of *in situ* determinations and to the predictions of a model. The direct measurements were all made near the Equator at a range of different times and by several different groups, all using versions of the 'grab-sampling' technique. The data are taken from ref. 12, and the model is the Oxford two-dimensional model¹³ which treats processes as a function of latitude and height but averages over longitude. The examination of several such comparisons¹⁴ implies that: (1) SAMS N_2O agrees quite well with *in situ* data near ~ 30 km but is some 30% higher below; (2) SAMS N_2O profiles are substantially smaller than the model predicts above 35 km, even when seasonal variability (the amplitude of which is shown in Fig. 5) is taken into account in both cases. The reason for the discrepancy (1) is unknown; further comparisons with more techniques are needed. The difference (2) is probably not dynamical in origin because similar comparisons for methane (like N_2O , CH_4 is fairly long-lived in the stratosphere) agree much better. The cross-section of atomic oxygen at ultraviolet wavelengths near

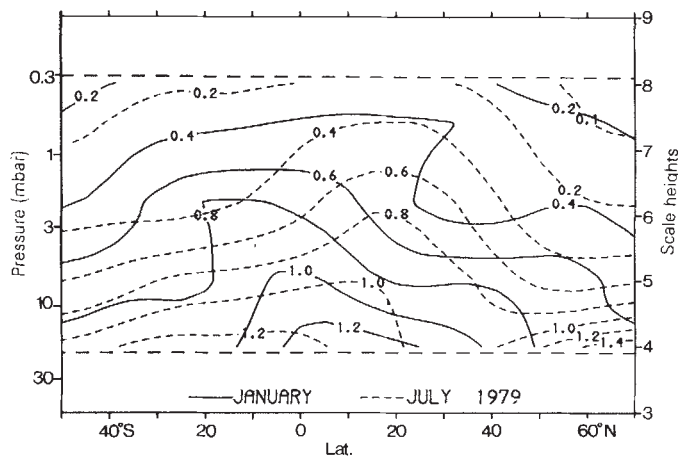


Fig. 6 Monthly mean latitude-pressure sections of CH_4 (p.p.m.v.) measured by the SAMS for the months January and July 1979. The differences seen are part of a seasonal trend which repeats each year, the maximum of methane concentration tending to follow the Sun but with a pronounced, height-dependent lag.

200 nm may be smaller than thought currently¹⁴. If so the model would underestimate the rate of photochemical destruction of N_2O in the upper stratosphere, as observed; hence the suggestion requires laboratory tests. The likelihood of a significant source of N_2O in the upper atmosphere, due, for example, to solar protons and relativistic electrons interacting with molecular nitrogen¹⁵ is reduced by these preliminary comparisons between measurement and theory.

Methane measurements by SAMS show very close agreement with *in situ* measurements and also with the two-dimensional model¹³. Figure 6 illustrates the global, zonally-averaged distribution of CH_4 for 2 months: January 1979 and July 1979. Methane has a photochemical time constant of the order of several months at 40 km and years at 30 km; the structure apparent in Fig. 6, which changes progressively from month to month, is, therefore, thought to result from dynamical effects. An obvious interpretation is that a poleward overturning of the stratosphere analogous to the tropospheric Hadley circulation occurs and is strongest in the summer hemisphere. Examination of a larger data set show that intermediate states with two equal low-latitude maxima occur around the equinoxes between the extremes in Fig. 6. This behaviour is not reproduced in our model and suggests a persistence of the summer circulation which is not predicted by any stratospheric model at present.

SAMS made water vapour observations by two different techniques: thermal emission measurements in the long-wavelength pure rotational band and observations of resonance fluorescence in the $2.7\text{-}\mu\text{m}$ vibration-rotation bands. The latter require very low collision rates between molecules and direct radiation from the Sun and hence apply only to the dayside mesosphere. The retrieved values¹⁶ are quite low, of the order of 1 part per million by volume (p.p.m.v.) or less, above 70 km. Below 65 km, the thermal emission measurements correspond to ~ 10 p.p.m.v., falling to around 4 p.p.m.v. below 35 km, where there are many other measurements for comparison; such comparisons confirm the values as typical of the lower stratosphere. The dry mesosphere and the sharp decline in humidity near 65 km is not surprising as photodissociation is an effective sink at these levels, although the actual values need validation. The large values in the upper stratosphere, however, do not agree with any model and if confirmed will require a further, presently unknown, source of water vapour, as methane oxidation alone cannot, according to models, raise the value above about 8 p.p.m. We assume that the retrievals from measurements in the rotation band have large uncertainties because the properties of the spectral lines, especially their shapes, and particularly for the self-broadened lines in the modulator cell, are not known well

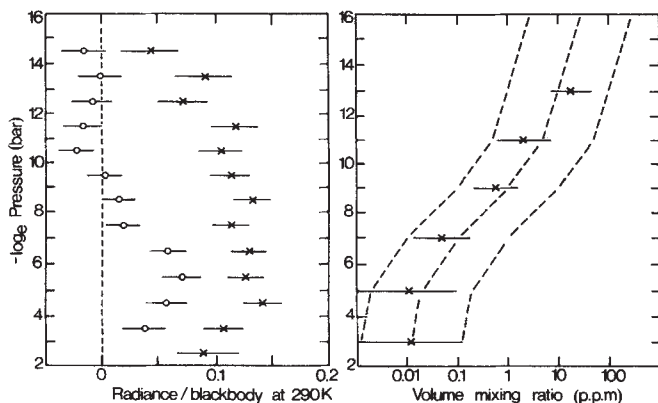


Fig. 7 *a*, Zonal mean profiles of radiance emitted by carbon monoxide; daytime (x) includes resonance fluorescence, nighttime (o) is only thermal emission. Average of data from 45° S to 45° N for the period of 25 December 1978 to 30 June 1979. *b*, Carbon monoxide profile derived from daytime radiances averaged over 35°–70° N for the same time period as *a*. Horizontal lines are error bars. The dashed lines are a first guess and its assumed standard deviation¹⁸.

enough. Furthermore, recent spectroscopic measurements of water vapour in the 2.7 μm band from Spacelab 1 suggest¹⁷ that the width of the lines at low pressure is greater than believed previously, a finding which may have consequences for the longer wavelength band, once its cause is understood. These aspects are being investigated theoretically and in the laboratory as part of the SAMS data validation programme.

The CO band and one of the CO₂ bands which SAMS observed (at 4.7 and 4.3 μm respectively) also emit strongly on the day side by resonance fluorescence (Fig. 7*a*). For CO₂ these data will provide information on the populations of the vibrational energy levels which relate to mesospheric heating and cooling rates¹. The CO radiances have been inverted to obtain abundance profiles¹⁸; (see Fig. 7*b*). The mixing ratio increases quite sharply above ~40 km due to a combination of two sources, photolysis of CO₂ and oxidation of CH₄. The latter is probably the major contributor in the stratosphere, but detailed model comparisons with the complete data set will be required to make a definite statement about the relative magnitudes of height-dependent sources.

The very important minor constituent ozone is not measured by SAMS, but several studies have been carried out on the relationship between ozone concentration and temperature. Barnett *et al.*¹⁹ used Nimbus 4 Selective Chopper Radiometer and Backscatter Ultraviolet Spectrometer (BUV) data (for tem-

perature and ozone, respectively) and found a strong negative correlation between the two in 1970. Recently, Keating²⁰ has used SAMS and BUV data to perform a similar study for 1980 which concluded that the temperature-ozone correlation coefficient had undergone a secular change. This is attributed tentatively to the change, during the 10-yr period, in the abundance of chlorine-containing species in the stratosphere due to the release of freons at the surface. Such a dependence had been predicted theoretically; it has been suggested that the order of magnitude of the change is about that expected²⁰.

Future activities

The completion of the above studies and the initiation of new ones relies on reprocessing, inversion and gridding of the large database using the latest algorithms which derive from the fullest possible understanding of how the instrument performed in space²¹. The validation of the retrievals of water vapour and intercomparisons with other methods of measurement are still in progress, the latter as part of the international Middle Atmosphere Programme.

Data from satellite experiments are acquired in such large quantities that archiving is a problem, particularly when access to the data set is needed by modellers, theoreticians and *in situ* and satellite experimenters who have no previous experience with the instrument which made the measurements. In the United States, the National Space Science Data Center (NSSDC) has been set up for this purpose at the Goddard Space Flight Center; SAMS data covering a 4-yr span are being archived there, both as calibrated radiances and gridded geophysical parameters. A similar archive is planned in the United Kingdom, probably at the Rutherford Appleton Laboratory, initially for Nimbus data but to accommodate eventually geophysical data from a wide range of UK experiments. A system offering remote access to the data from a network of points in the United Kingdom is under consideration.

The next opportunity for acquiring long-term coverage of the middle atmosphere from a satellite should be the launch of the NASA Upper Atmosphere Research Satellite in 1989. One instrument on board this new class of space shuttle-launched satellite is the Improved Stratospheric and Mesospheric Sounder which uses helium pressure modulators to drive miniature Stirling cycle coolers to run the detectors and their fore-optics at 80 K. The improved sensitivity (up to two orders of magnitude) allows the use of higher vertical resolution and the identification and study of local perturbations in temperature and composition and their inter-relationships, while preserving the global, diurnal and seasonal coverage which makes the satellite such a powerful technique for the study of atmospheric chemistry, radiation and dynamics.

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Preliminary Phanerozoic polar wander paths for the North and South China blocks

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Polar wander paths for the North and South China blocks suggest that (1) both were parts of Gondwana in the Palaeozoic, (2) the North China block accreted to Siberia in the late Permian and (3) the South China block accreted to the North China block in the middle Triassic to the early Jurassic. Comparison of the polar wander path for the South China block with that for northern Eurasia suggests that relative motion of over 4,000 km has occurred between them.

CHINA, like the rest of Asia, is composed of a number of tectonic blocks or cratons, separated by fold belts which mark the sutures where they have been welded together. Palaeomagnetism can be used to elucidate the origin and accretionary history of the blocks and is therefore important in geological studies of Asia¹⁻⁶. We have made a detailed palaeomagnetic study of the North and South China blocks (NCB and SCB) to determine their apparent polar wander (APW) paths.

Regional geology and sampling

Geologically, the NCB is separated from Siberia by the Central Asian fold belts in the north and from the SCB by the Qinling fold belt in the south. The basement of the NCB consists of Archaean and early and middle Proterozoic metamorphic rocks. With the Luliang orogeny (~1,800 Myr) the parts of the NCB were welded together to form a cratonic block. We collected samples from Shandong, Liaoning and Shanxi provinces and Jixian County (Fig. 1) and found that almost all of the periods from the Tertiary to the middle Proterozoic are represented. We collected a total of 1,981 samples from 250 sites but report here only the Phanerozoic results.

The SCB (the Yangtze paraplatform) is surrounded by fold belts, such as the early Palaeozoic South China fold belt to the south east and the Qinling fold belt to the north. It is connected to the NCB by the Huaiyang Shield in the north east. Most of the basement of the SCB is late Proterozoic. With the Jinning orogeny (~850 Myr) the SCB completed its transition from fold belt to cratonic block. We collected samples from Zhejiang, Guizhou, Yunnan and Hubei provinces (Fig. 1) representing all the periods from the Tertiary to the late Proterozoic, the age of the oldest unmetamorphosed rocks in the SCB (1,029 samples from 180 sites).

Our samples were measured on cryogenic and spinner magnetometers using alternating field and thermal demagnetization methods. As many sites were demonstrably remagnetized, Zijderveld diagrams, vector differences and remagnetization circle methods were also used. All the results from this study and the few previous results satisfying the minimum criteria of reliability^{4,5} are listed in the China palaeomagnetic data list⁷, including 69 NCB and 90 SCB poles. The pole positions from our study are reported from sites, each consisting of five independently-oriented samples (data list available from the authors). The APW paths for the SCB and NCB (Fig. 2a, b) are constructed from the data in Table 1.

Palaeopoles from the SCB

Our Tertiary poles consist of determinations from three late Miocene basaltic lavas in Zhejiang Province (South China (SC) 1-3) giving a mean pole (SC 4). The results include antipodal normal and reverse flows, but with so few flows we cannot estimate how well secular variation has been averaged. The results, however, are consistent with studies of red beds in Hunan Province³, which also pass a reversal test. Thus, whereas the

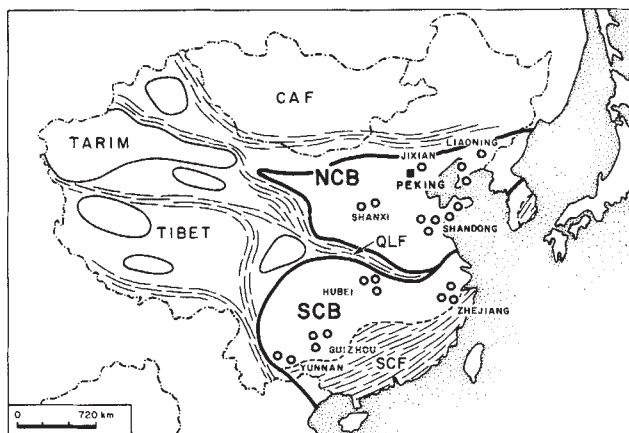


Fig. 1 Site map with major tectonic units in China shown schematically. The solid lines are block boundaries; CAF, Central Asian fold belts; QLF, Qinling fold belt; SCF, South China fold belt.

Tertiary APW path clearly is not well constrained, it may include a loop towards northern Europe.

The Cretaceous results are from widely separated parts of the SCB; the Upper Cretaceous pole (SC 7) is for South Korea⁸. Our seven Lower Cretaceous sites in volcanic and sedimentary rocks are in Zhejiang, giving the mean pole SC17, consistent with SC 8 (ref. 9) and SC 9 (S. Sasajima, personal communication).

The mean Upper Jurassic pole (SC 24) from this study is based on six poles, SC 18-23, in Zhejiang ignimbrites and sandstones. The Middle Jurassic result (SC 27-30) from red beds in Guizhou province includes normally and reversely magnetized samples. Results from Hong Kong (SC 25) (S. Sasajima, personal communication) and Anhui province (SC 26) (ref. 10) are also included.

The Middle Triassic pole SC 32 (Chan Lung-san, personal communication) is preliminary, being based on eight samples from two sites, but the results do include two polarities and pass a fold test.

A collection of 42 samples from 13 sites in the Changxing carbonate section, a Permian/Triassic type section, yielded a consistent direction after thermal demagnetization to 300 °C. The resultant mean pole is SC 46, close to that obtained for the Middle Triassic.

Most of the 56 Permian samples studied from 10 sites in the Emeishan basalts of Guizhou carried a late Mesozoic or recent characteristic direction. Their magnetization was complex with at least four directions. Three of these are clearly secondary,

Table 1 Mean palaeopoles for the North and South China Blocks

Age	P _{lat.}	P _{long.}	K	A ₉₅	DP	DM	N	Test	China List
SCB									
TM-TP	74.2	36.5	148	7.6			8	R	SC 1-5
KU	69.0	191.1	85	8.3	10.1	12.9	5		SC 7
KL	76.2	225.7		4.8			14	R	SC 8-17
JU	73.0	213.7	29	12.6			6		SC 18-24
JM	68.8	202.1		14.0			9	R	SC 25-30
TRM	54.6	209.7	95	(5.7)	3.3	6.2	2	RF	SC 32
PU-TRL	50.7	204.8	38	6.8	5.4	8.6	13		SC 33-46
PU	29.3	235.3	41	(12.0)	7.4	13.4	1	R	SC 47
CU	21.5	224.6	28	17.8	8.9	17.8	4	RF	SC 54-59
CL	25.2	220.7	12	(28.2)	14.3	28.4	1		SC 61
CBL	3.4	195.0	28	8.8			8	RA	SC 64-72,78
NCB									
TM	76.4	178.0	230	6.0	7.2	9.3	4	R	NC 1-5
KU	69.0	182.0		4.0			8		NC 6
KL	69.0	200.0	40	12.2	12.5	17.5	5	R	NC 7-12
JU	71.3	225.7	38	7.9	6.7	10.3	10		NC 13-23
JU	74.2	215.6	57	4.0	3.7	3.4	10		NC 24
JM-JU	72.1	202.0	194	6.6	6.8	9.5	4		NC 25-29
JM	81.9	253.3	41	9.6	8.4	12.6	4		NC 30
TRL	35.3	39.3		17.6	14.0	22.0	6	R	NC 35-39
PU	38.1	6.3					2	R	NC 40-42
PU	44.0	358.0	40	6.9	3.8	7.2	12	R	NC 43
OM	43.2	332.5	53	(10.6)	5.3	10.6	1	R	NC 44
CBL	15.0	298.6	38	9.9			7	R	NC 46-60
PCU	16.5	301.1	35	9.5	8.8	12.9	8	R	NC 61-69

The database and statistics of the APW paths for the NCB and SCB. Column 1, age; columns 2 and 3, pole position; columns 4 (*K*) and 5 (*A*₉₅), precision parameter and 95% error in Fisher's³⁹ analyses; columns 6 (*DP*) and 7 (*DM*), semi-axes of the elliptical error around the pole at 95% probability, *DP* in the colatitude direction and *DM* perpendicular to it; column 8 (*N*), number of sites; column 9, stability tests, R for reversal test, F for fold test and A for attitude test; column 10, pole number in China palaeomagnetic data list⁷. Abbreviations: TM-TP, Tertiary Miocene-Pliocene; KU, Upper Cretaceous; KL, Lower Cretaceous; JU, Upper Jurassic; JM, Middle Jurassic; TRM, Middle Triassic; PU-TRL, Upper Permian-Lower Triassic; CU, Upper Carboniferous; CL, Lower Carboniferous; CBL, Lower Cambrian; OM, Middle Ordovician; PCU, Upper Precambrian.

being in the present dipole field direction and in the Jurassic normal and reversed directions. We tentatively identify the fourth as a primary Permian direction which gives the pole SC 47, approximately antipodal to the Permian/Triassic boundary result. A recently-reported Permian pole for the SCB⁶ is inconsistent with our result. It may be a secondary remagnetization⁷, or the result of tectonic rotation (M. W. McElhinny, personal communication), so is not included in the APW path described here.

The Upper Carboniferous poles (SC 54-59) were obtained from reversely magnetized samples, consistent with a Kiaman reversed interval age. The samples were from a fresh man-made outcrop and were not as severely remagnetized as the remaining Carboniferous sites. The Lower Carboniferous pole (SC 61) was obtained from normally magnetized results. Thus, our Carboniferous results are based on data which pass a reversal test. They also pass a fold test.

We did not obtain useful data from Devonian, Silurian or Ordovician sites, all of which were remagnetized strongly in Recent or Mesozoic directions.

The Lower Cambrian poles are based on collections from Zhejiang, Hubei and Yunnan provinces. The poles from the Hetang Formation in Zhejiang and the Tianheban Formation almost 1,000-km away in Hubei satisfy an attitude test at the 95% confidence level but are discrepant before bedding correction. The Tianheban is flat lying. After the Hetang Formation results have been corrected for early Palaeozoic tilt, the data from both formations are in good agreement, this test being comparable with a fold test.

Palaeopoles from the North China block

The Tertiary poles (North China (NC) 1-5) include both normal and reversed polarities, but are preliminary because they are both from the same area (Linju County of Shandong Province) and from basalts. The Upper Cretaceous pole (NC 6; ref. 11) and the Lower Cretaceous poles (NC 7-12) are from andesites

and rhyolites in Zhuji County of Shandong. The Upper Jurassic sites (NC 13-23) are in sandstone of the Laiyang Formation in Zhuji County of Shandong, consistent with (NC 24; ref. 10), obtained from the Mengying Formation in western Shandong. The Middle/Upper Jurassic poles (NC 25-29) are for siltstones from quarry sites of the Santai Formation in Zibo of Shandong. In all these studies, alternating field and thermal demagnetization methods gave consistent results.

Preliminary Lower Triassic results from red beds in three areas have similar secondary magnetizations. (The results from Zibo, Shandong Province are so close to the present field direction before bedding correction that they are not included.) Two sites from Taiyuan of Shanxi Province give scattered results with a northwesterly declination and shallow positive inclination. The four sites from Benxi of Liaoning Province include both normal and reversely-magnetized rocks. The normal samples have a similar direction to those from Shanxi Province. The direction in reversely-magnetized samples migrates towards that antipodal to the normal samples on thermal demagnetization, but even at high temperature fail to reach the antipodal direction. The poles from Liaoning Province are included (NC 35-39).

The Upper Permian poles (NC 40-42) and (NC 43; ref. 6) are from a Permian/Triassic sedimentary section in Taiyuan of Shanxi. Sandstones in the section are heavily remagnetized, but mudstones gave consistent reversed directions after thermal demagnetization.

Most Ordovician samples were remagnetized so strongly that no useful information could be obtained; the Middle Ordovician pole position reported here (NC 44) is from only one site but is based on AF and thermal demagnetization, hence we tentatively suggest that it is an Ordovician characteristic direction.

The Lower Cambrian pole positions (NC 46-56) and (NC 58-60) from oolitic carbonate in Zhangxia of Shandong and from carbonate in Fuzhouwan of Liaoning have different thermally-cleaned directions from any observed Phanerozoic

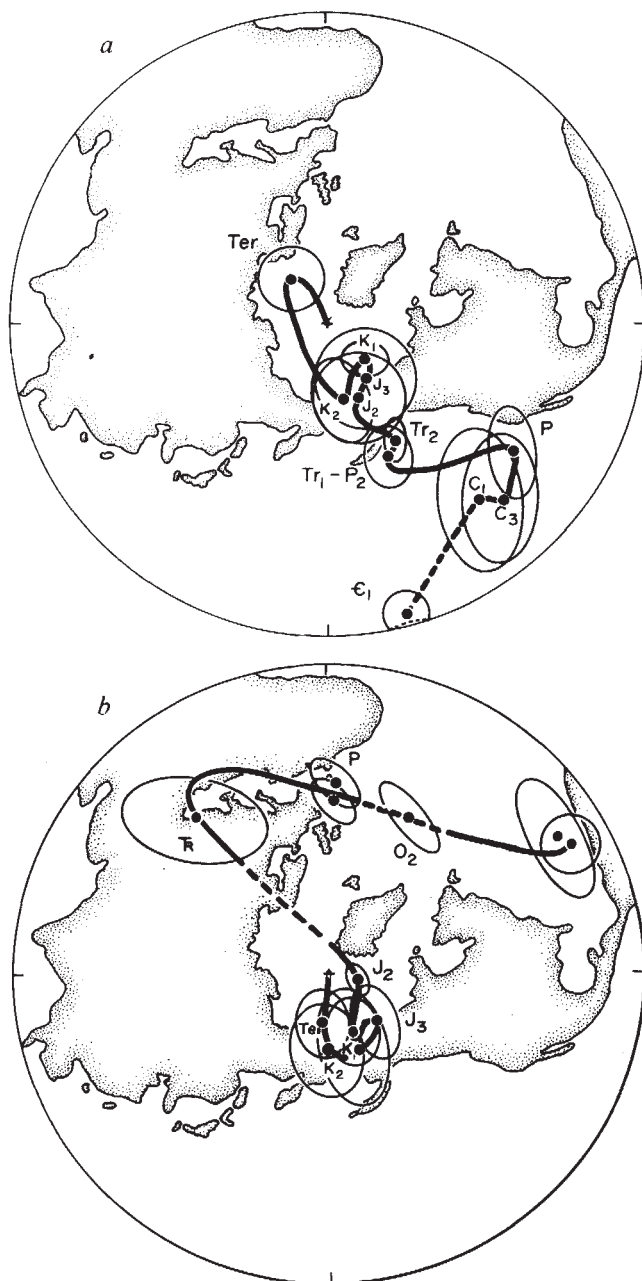


Fig. 2 *a*, APW paths for the SCB; *b*, the NCB. Ter, Tertiary; K₂, Upper Cretaceous; K₁, Lower Cretaceous; J₃, Upper Jurassic; J₂, Middle Jurassic; Tr₂, Middle Triassic; Tr₁, Lower Triassic; P₂-Tr₁, Upper Permian to Lower Triassic; P, Permian; C₃, Upper Carboniferous; C₁, Lower Carboniferous; O₂, Middle Ordovician; E, Cambrian; E₁, Lower Cambrian. Circles and ellipses are the A₉₅ or α₉₅ confidence intervals of pole position. The poles used in construction of the two APW paths are indicated in Table 1, where the relevant statistics are also given.

characteristic directions. Lower Cambrian sandstones in North Korea have a similar direction (NC 57; ref. 11). We therefore suggest that this is a Lower Cambrian characteristic direction. The pole positions from the Upper Precambrian Nanfen Formation (NC 61-69) are very close to the Lower Cambrian pole position, supporting the suggestion that the Nanfen Formation is close to early Cambrian in age.

Summary of palaeomagnetic results

The APW paths can be traced back unambiguously to the Carboniferous for the SCB and to the Permian for the NCB but the middle Palaeozoic poles require much more work on both blocks. In contrast, the Cambrian poles are well established for

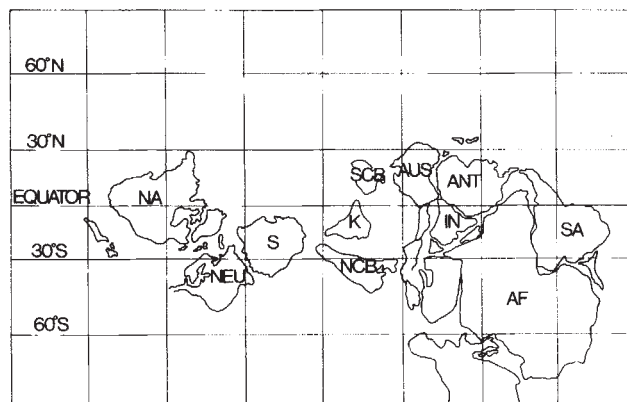


Fig. 3 Lower to Middle Cambrian reconstruction modified from ref. 13. AF, Africa; ANT, Antarctica; AUS, Australia; IN, India; K, Kazakhstan; NA, North America; NEU, northern Europe; S, Siberia; SA, South America. Note that the NCB and SCB are treated as separate blocks.

each block. Because of the absence of middle Palaeozoic data, there is a polarity ambiguity which is resolved by assuming that the polar wander between the age of the oldest reliable late Palaeozoic results and the Cambrian was less than 90°.

When compiling the APW paths for the major continental blocks, palaeopoles have been selected according to four criteria: demagnetization, statistics, the accuracy of ages and possible tectonic rotations¹². All our results (Table 1) were based on either thermal or alternating field demagnetization, or both methods. With the exception of SC 061, which has a 95% confidence radius of 28.2°, all the results in Table 1 are based on palaeopoles having confidence intervals <25°. Most of our samples were collected from stratigraphic type sections for which there is good fossil control. The ages of the Phanerozoic units sampled are usually elucidated at stage level and at least to the series (that is, Lower, Middle and Upper) level in terms of the geological time-rock units, so that their ages are generally known to better than 25 Myr. As we sought to obtain APW paths for the cratonic NCB and SCB, we collected neither from fold belts nor from tectonically-active areas near the margins of the two blocks. These data therefore satisfy standard palaeomagnetic criteria (for example, ref. 12).

In addition, reversal and fold or attitude tests are satisfied by some of the sites (Table 1). APW paths for the NCB and the SCB therefore should be sufficiently reliable to begin to test tectonic hypotheses, although they will be refined by later data.

Cambrian palaeogeography

Based on the palaeomagnetic results, we propose that: (1) the NCB and SCB were separate in the Cambrian because their palaeopoles are quite different; (2) the SCB is placed adjacent to northern Australia, occupying an equatorial latitude, whereas the NCB is placed near to Tibet, northern India and Iran at 35° latitude in the Southern Hemisphere (Fig. 3). Thus both the NCB and the SCB were parts of Gondwana, but were separated from each other.

The proposed position of the SCB has the following merits: (1) the strong affinity of the Cambrian trilobites between the SCB and Australia is explained readily¹⁵⁻¹⁷; (2) the sedimentary facies belts in the SCB and Australia match each other, for example, the sequence from the west to the east is, first, a clastic belt, then the miogeosynclinal facies belt of shallow marine carbonate and finally, the eugeosynclinal facies belt of deep-marine cherts or flysch facies^{14,17,18}; (3) the huge phosphorite deposits in Yunnan Province and in North Vietnam of early Cambrian age and those of middle Cambrian age in Hainan Island of the SCB and the Georgina Basin of Australia would all have been formed adjacent to each other in Cambrian tropical marine basins; (4) archaeocyathids in the early Cambrian and gypsum and salt pseudomorphs in middle and late Cambrian

of the SCB and Australia are consistent with the tropical and low latitudes derived from the palaeomagnetic results.

The Southern Hemisphere position for the NCB depends on interpretation of the polarity of its Cambrian pole. It is supported by the distributions of the Cambrian trilobites¹⁵ and early Ordovician cephalopods¹⁹, as well as the well-documented Cambrian evaporite belt from the Arabian Peninsula via Iran, Pakistan, northern India to North-west and North China²⁰⁻²². This all suggests that in the early Palaeozoic the North China block was close to Tibet, northern India and Iran (see ref. 7).

Permian reconstruction

The late Permian palaeomagnetic results from the NCB show that it was then very close to the equator; the Permian reconstruction described here, with the Chinese blocks in Tethys, next to northeast Africa and the Arabian Peninsula (Fig. 4), is different from previous reconstructions^{13,23,24}.

The merits of this new reconstruction are: (1) it satisfies the low palaeolatitudes of the Chinese blocks suggested by Permian fossils; (2) it explains the Permian flora distribution, for example, the four flora provinces of Angara, Eurameria, Gondwana and Cathaysia are all grouped together and at the appropriate latitude in this reconstruction; (3) it is consistent with the tectonic history of the region, for example, the Central Asian fold belt was sutured finally in the late Permian by a process which started in the west and propagated eastwards. This is the so-called 'migration of geosyncline'²⁷, which we interpret as the collision of the NCB with the Siberian block starting from Northwest China and extending to Northeast China (see ref. 7).

Assembly of the China blocks

The Cambrian poles from Shandong and Liaoning provinces of North China are different from the Zhejiang, Yunnan and Hubei provinces of South China (Table 1), demonstrating that the NCB and SCB were distant from each other. In contrast, the Middle Jurassic pole from Shandong Province of the NCB and those from Zhejiang Province of SE China and Guizhou Province of SW China are statistically indistinguishable, implying that the NCB and SCB were assembled as a single unit by the middle Jurassic⁷.

The Permian results from Shanxi Province show that the NCB had an equatorial latitude in the late Permian. Similarly, the late Permian basalt direction in Guizhou Province shows that the SCB was also in an equatorial region during that period. The relative orientations of the two blocks, however, were quite different in the Permian from those at present, suggesting that although they were close to each other in the Permian, they were not welded into a single tectonic unit. The palaeomagnetic results are consistent with the evidence from palaeontology and sedimentary facies^{28,29}. We have no detailed palaeomagnetic results from Middle Triassic to Lower Jurassic for either blocks, preventing accurate timing of the amalgamation of the NCB and SCB; analyses of sedimentary facies in the two blocks²⁸ suggests that the amalgamation occurred during the Triassic Indosinian orogeny.

Eastward movement of the SCB

The palaeomagnetic pole positions from the NCB and SCB are displaced to the east of the northern Eurasia path (Fig. 5a). Nevertheless, the Mesozoic segments of the APW paths for the SCB and northern Eurasia are similar in form. Rotation of the former clockwise by 50° about an Euler pole near the present north pole brings the two paths into agreement (Fig. 5b). Such a rotation brings the SCB westward to the region presently occupied by western India and Pakistan, suggesting that relative motion of about 4,000 km occurred between the SCB and northern Eurasia. The SCB is thought to have been pushed eastward during the Cenozoic when the Indian plate collided with the Eurasian plate in the Eocene and subsequently as it continued its northward motion^{30,31}. Our palaeomagnetic data suggest a far larger displacement than the proposed eastward motion, which is only several hundred kilometres.

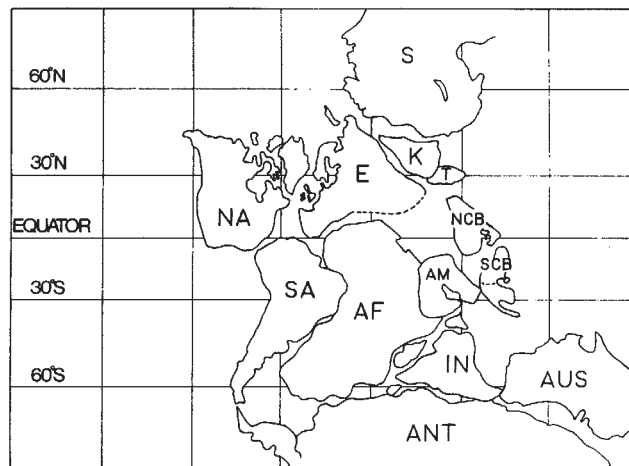


Fig. 4 Permian reconstruction. AM, Asia Minor; E, Europe; I, India; T, Tarim; remaining abbreviations as Fig. 3. The grouping of Gondwana and Laurasia is adopted from the Pangaea A^{25,26}. The relative configuration between Gondwana and Laurasia and the positions of the Chinese blocks are based on the Pangaea B²⁶. Note that although the Tarim block may have been accreted already to the Kazakhstan block so that they share the same Angara flora, the NCB and SCB were still distant from the Siberian block. The accretion of the NCB to the Siberian occurred in the late Permian.

We sought to establish whether the difference between the APW paths of South China and northern Eurasia is statistically significant. We consider the segments of the two APW paths from the Carboniferous to late Cretaceous. The APW path segment for the SCB consists of nine poles, that is poles of K2, K1, J3, J2, TR2, P2-TR1, P2, C3 and C1. They all fall to the east of the corresponding poles from northern Eurasia, which is highly improbable (1 in 512) if the two groups of poles are statistically indistinguishable. The 95% confidence intervals for the palaeopoles from both northern Eurasia and South China are for the most part <15°. Using these values, there is overlap between the confidence intervals of some of the Mesozoic poles of the two paths. Using the standard errors ($P = 0.63$) of other palaeomagnetic workers when comparing APW paths¹², however, there is no overlap. The two curves are therefore statistically distinguishable.

The inclination data and the implied palaeolatitudes of the SCB show that the observed difference in the northern Eurasia and SCB APW paths does not result from a local rotation (Fig. 6). Assuming no relative motion between the SCB and northern Eurasia, the palaeolatitude is significantly lower at Hongzhou than that from the northern Eurasia APW path. Thus the difference in APW paths cannot be owing to a local rotation, but must involve substantial relative motion between the two regions. The palaeolatitudes become statistically indistinguishable for westward rotation of the SCB by 45, 50 or 55° about an Euler pole close to the north pole with a best fit of 45° for the last 75 Myr, whereas a rotation of more than 55° is needed to fit the segments before 225 Myr. Therefore, there may have been two stages of relative movement between South China and northern Eurasia.

The amount of relative motion along the boundary between two blocks is very dependent on the distance from the Euler pole, reaching a maximum when the pole is 90° from the boundary. Fitting the Mesozoic path segments gives an Euler pole at 86.5N31.5E with angle of rotation 50° clockwise and a displacement of $4,300 \pm 1,200$ km eastward for the SCB path with respect to the northern Eurasia path.

We conclude from the analysis of the APW paths that the SCB has moved eastward with respect to northern Eurasia by ~4,000 km. Two fundamental questions remain: first, as the SCB and Siberia are separated presently by 2,500 km, how were the 4,000 km of relative motion distributed between the intervening tectonic units? Second, when did this motion take place?

The Middle Jurassic poles from the NCB and SCB are statistically indistinguishable, differential motion towards this pole cannot therefore have been more than 1,500 km since the middle Jurassic, using the 63% errors of these poles. This suggests that a major portion of the relative motion between the SCB and northern Eurasia occurred along the Central Asian fold belt forming the boundary between the NCB and northern Eurasia. This fold belt is the largest in the world; it is aligned approximately east-west and is still active^{32,33}. It has been considered already as a possible plate boundary between the seismically-active Chinese blocks and the aseismic northern Eurasia plate³⁴. We consider that much of the relative motion, required by the APW paths to have occurred between the SCB and northern Eurasia, may have been along this Central Asian fold belt.

It is tempting to assign the relative motion to the Cenozoic and to interpret it as a consequence of the Indian-Eurasian continental collision. First, a single rotation brings the two Mesozoic APW paths into agreement. Second, the relative motion suggested by the palaeomagnetic data is just equal to the width of the northern India (3,000 km) plus the width of the transverse ranges between India and the SCB (1,000 km), consistent with a rigid indenter displacing parts of the southern boundary of Asia along previously established lines of weakness. Our data, however, do not preclude motion between the SCB and northern Eurasia during the Mesozoic because of the inherent limits of the resolution of the palaeomagnetic method. We do know that the NCB rotated substantially with respect to both Eurasia and the SCB during the Triassic. Our interpretation is that relative motion of at least 2,500 km occurred between the NCB and northern Eurasia during Cenozoic, possibly along the Central Asian fold belt in addition to the southern belts^{30,31}.

Accretionary history of China

Our study has revealed a complicated geological history for the NCB and SCB. In the Cambrian, both blocks seem to have been part of Gondwana, but were separated from each other. The SCB had an equatorial palaeolatitude and was near northern Australia whereas the NCB was at 35° S, close to Iran, Tibet and northern India. During the period from the late Ordovician to Carboniferous, Gondwana moved across the South Pole, causing an inversion of the latitude zones^{13,35}. The NCB then led the SCB in their common northward movement. In the late Carboniferous and early Permian, the NCB arrived near the palaeoequator; in the Permian, the NCB was in Tethys, next to the Iranian, Turkish and Arabian blocks. The SCB had become separated from Australia by the Devonian and moved toward the NCB. In the late Carboniferous and early Permian, both blocks were in Tethys with the Indochina block. During the late Permian, the NCB started to move northward and collided with Siberia along the Central Asian fold belt. It was not until the middle Jurassic, however, that the NCB and SCB were welded together. Since the beginning of the Cenozoic, when the Indian plate moved northward and collided with the Eurasian plate, the Chinese blocks have been pushed further eastward. In particular, the SCB has moved eastward with respect to northern Eurasia.

The following sequence is suggested: (1) Kazakhstan accreted to Siberia at the end of the Carboniferous³⁶; (2) Russia collided with Siberia in the early Permian³⁷; (3) the NCB accreted to Siberia in the late Permian⁷; (4) the SCB collided with the NCB by the middle Jurassic, possibly during the late Triassic; (5) central Iran accreted to Eurasia in the early Cretaceous; (6) India collided with Eurasia during the Eocene³⁰; (7) the Arabian Peninsula separated from Africa and has moved toward Asia since the late Tertiary (15 Myr); (8) 50 Myr in the future, Australia may collide with Eurasia. It thus seems that Asia has grown by accreting fragments from Gondwana. Conversely, these fragments have been drifting away from Gondwana for the past 250 Myr. This remarkably long history of rifting around Africa may result from insulation³⁸ beneath the largely stationary core of Gondwana, which produces the necessary heat accumulation to drive the rift systems.

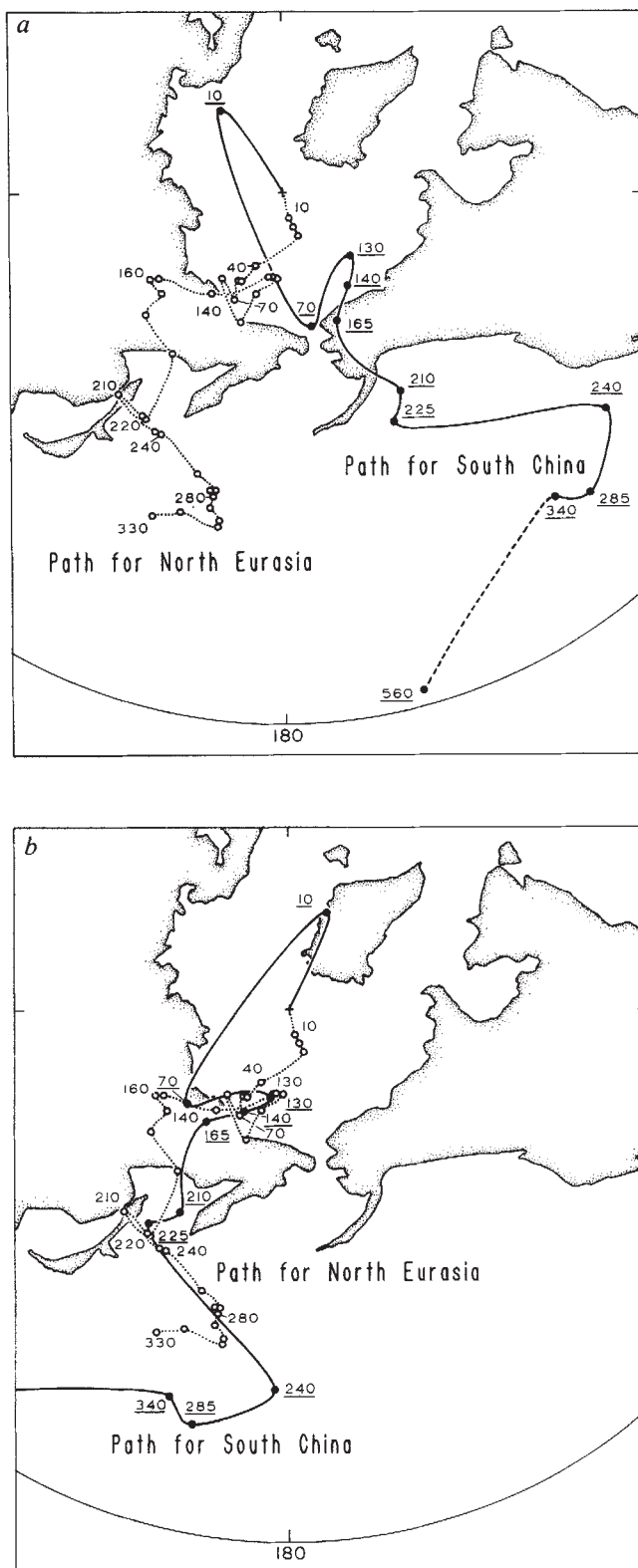


Fig. 5 Comparison of the APW paths for northern Eurasia¹² (dotted line), and for the SCB (solid line). Open circles, pole positions at 10 Myr intervals from northern Eurasia; solid circles, those from the SCB; numbers, suggested ages of the poles (Myr). The ages for the poles of the SCB are underlined. *a*, The South China APW path is displaced to the east with respect to the northern Eurasia path. *b*, A 50° clockwise rotation about an Euler pole near the present north pole brings the Mesozoic and late Palaeozoic segment of the Chinese path into agreement with that of the northern Eurasia path. The same rotation brings the SCB westward to a position presently occupied by western India and Pakistan.

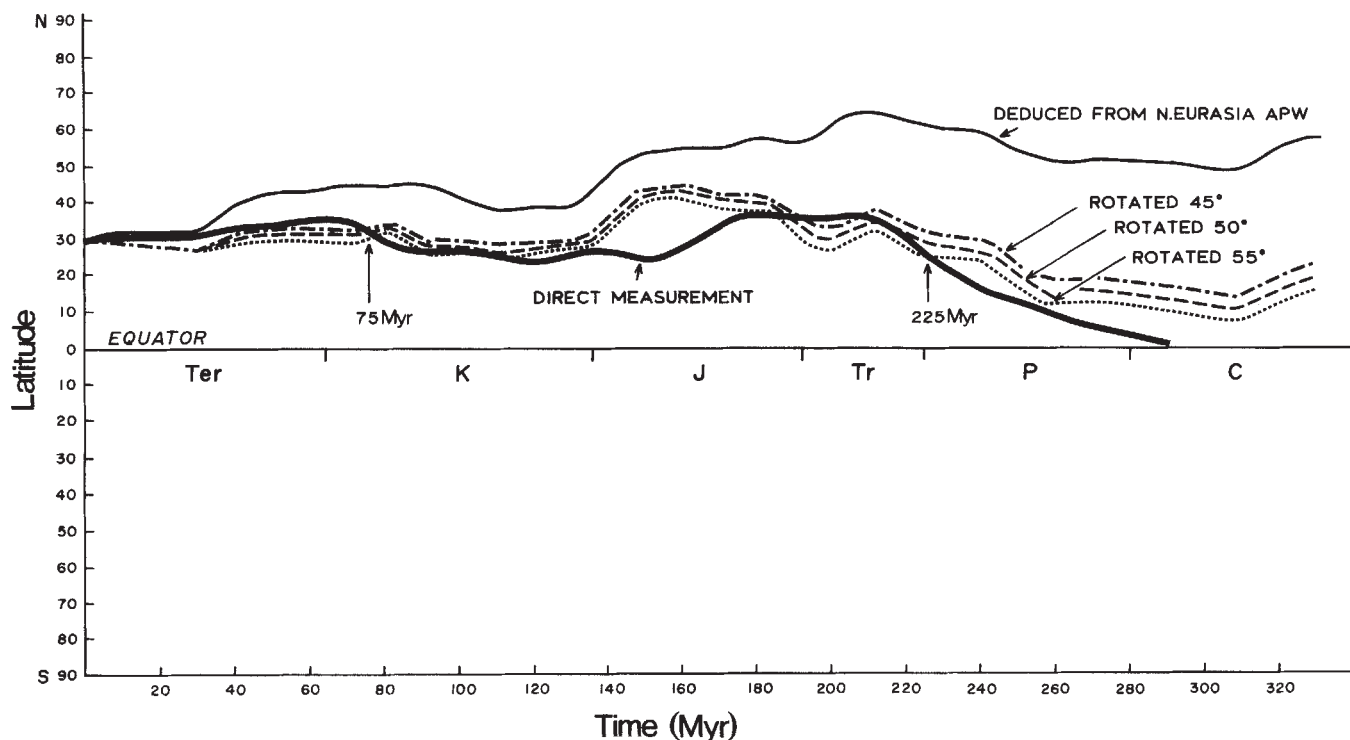


Fig. 6 Palaeolatitudes of the Hongzhou in the SCB determined from the South China samples (heavy solid curve) and those deduced from the northern Eurasia APW path (remaining curves). The uppermost curve shows the palaeolatitudes deduced from the northern Eurasia APW path with the SCB at its present position; the rest are those deduced with the SCB rotated westward by 45, 50 and 55°, respectively.

Conclusions

Preliminary APW paths for the NCB and SCB provide the palaeomagnetic constraints for these blocks in palaeogeographic reconstructions; the palaeomagnetic results from these blocks differ from those of Siberia and Europe and the Chinese APW paths are displaced systematically to the east of the northern Eurasia APW path. This eastward shift of the South China APW path suggests that the SCB has moved eastward 4,000 km with respect to Siberia, but the details of when and how this motion took place are as yet unknown.

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Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus

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The 9,213-nucleotide structure of the AIDS/lymphadenopathy virus has been determined from molecular clones representing the integrated provirus and viral RNA. The sequence reveals that the virus is highly polymorphic and lacks significant nucleotide homology with type C retroviruses characterized previously. Together with an analysis of the two major viral subgenomic RNAs, these studies establish the coding frames for the gag, pol and env genes and predict the expression of a novel gene at the 3' end of the genome unrelated to the X genes of HTLV-I and -II.

ACQUIRED immune deficiency syndrome (AIDS) is a novel, transmissible deficiency of cellular immunity characterized by opportunistic infections and certain malignancies, notably *Pneumocystis carinii* pneumonia and Kaposi's sarcoma, in patients without another recognized cause for contracting these rare diseases¹⁻³. AIDS is manifested by a profound lymphopenia, a generalized cutaneous anergy and a markedly reduced proliferative response to mitogens, antigens and allogeneic cells, seeming to result from depletion of the OKT4⁺ T-lymphocyte subset⁴. While humoral immunity is relatively unaffected, there is increasing evidence for a hyperactive B-cell proliferative response which may be related causally to the high incidence of B-lymphoma in AIDS patients^{5,6}. In addition to the fully-developed syndrome, an epidemic of a related disease, AIDS-related complex (ARC), has appeared, characterized by generalized chronic lymphadenopathy. This syndrome shares many of the epidemiological features and immune abnormalities and often precedes the clinical manifestations of AIDS. The predominant risk groups for AIDS and ARC include homosexually active males, intravenous drug abusers, recipients of transfusions and blood products and the heterosexual partners and children of high-risk individuals, suggesting the involvement of an infectious agent transmitted through intimate contact or blood products.

Recent evidence has implicated strongly a novel lymphocytotropic retrovirus as the primary aetiological agent of AIDS and the AIDS-related complex. Lymphadenopathy-associated virus (LAV) was isolated initially from cultured lymph-node T cells of patients with lymphadenopathy and AIDS as well as an AIDS patient and an asymptomatic sibling, both with haemophilia B⁷⁻⁹. A similar virus, designated human T-lymphotrophic virus type III (HTLV-III), has been isolated from a large number of AIDS and ARC patient blood samples by co-cultivation with the permissive T-cell line H9 (refs 10, 11). LAV and HTLV-III, as well as related retroviruses isolated recently from AIDS patients^{12,13}, share several important characteristics. Viral replication occurs in the OKT4⁺ T-lymphocyte population *in vivo* and *in vitro* and is associated with impaired proliferation and the appearance of cytopathic effects^{8,10,14}. The virus has a Mg²⁺-dependent reverse transcriptase, exhibits a dense cylindrical core morphology similar to type D retroviruses^{8,13,15} and is recognized by antibodies found in the sera of virtually all AIDS and ARC patients^{8,13,16-21}.

As a first step towards characterizing the molecular biology of this virus, we have determined the entire nucleotide sequence for one of the integrated proviruses present in H9/HTLV-III cells and for a complete set of overlapping complementary DNAs representing the viral RNA of distinguishable isolate(s) also present in H9/HTLV-III cells. Our results establish that

LAV/HTLV-III has no nucleotide homology with previously characterized animal and human retroviruses and that different virus isolates display significant genetic heterogeneity. Together with transcriptional mapping data, these studies provide a detailed picture of the structure and processing of the gag, pol and env gene products, provide evidence for a novel gene in the 3' region of HTLV-III and predict a further gene product in the region between the pol and env genes.

Isolation of cDNA and provirus clones

Molecular clones of HTLV-III were identified initially from cDNA libraries representing cellular RNA of productively infected H9/HTLV-III cells, established by Popovic *et al.*¹⁰. The H9 human T-cell line is permissive for the continuous production of high titres of HTLV-III isolated from the cultured lymphocytes of AIDS and ARC patients and is significantly resistant to the cytopathic effects of these viruses. The virus produced by H9/HTLV-III retains its cytopathic activity against fresh normal human lymphocytes¹⁰. Total poly(A)⁺RNA was prepared from H9/HTLV-III cells infected with pooled material from several different AIDS patients¹⁰ and used to construct an oligo(dT)-primed cDNA library in the vector λ gt10.

The strategy used to identify clones containing HTLV-III sequences was based on differential hybridization with cDNA probes prepared from poly(A)⁺RNA of H9/HTLV-III cells and the uninfected CEM human T-lymphoblastoid cell line; ~0.2% of the clones in this library contained inserts hybridizing specifically with the H9/HTLV-III cDNA probe. Six of these clones were purified and their DNA inserts used as probes to classify 50 additional H9/HTLV-III-specific clones. Two distinct classes of clones were identified on the basis of their pattern of hybridization; furthermore, weak hybridization was detectable between the two different classes. Inserts from one clone of each H9/HTLV-III-specific class (H9c.7 and H9c.53) were subcloned into phage M13 vectors and their sequences determined by the dideoxy-chain terminator method. Significantly, a 76-nucleotide sequence was shared by the 5' end of H9c.7 and the 3' end of H9c.53, accounting for hybridization between the two classes. The 3' point of divergence of this 76-nucleotide sequence was marked by a polyadenylate tract in H9c.53, as expected for an RNA polymerase II transcript. The congruence exhibited by the opposite ends of these clones was very like the terminal redundancy of the viral genome of retroviruses²²⁻²⁴, suggesting that clones H9c.7 and H9c.53 represented the 5' and 3' regions, respectively, of HTLV-III (Fig. 1).

The identity of H9c.7 and H9c.53 was confirmed by blot hybridization analysis of H9/HTLV-III and normal human lymphocyte genomic DNA restriction digests. Sequences hybridizing with H9c.7 and H9c.53 were found only in DNA from infected cells, demonstrating their exogenous viral origin (Fig. 2A). To determine whether related sequences are associated

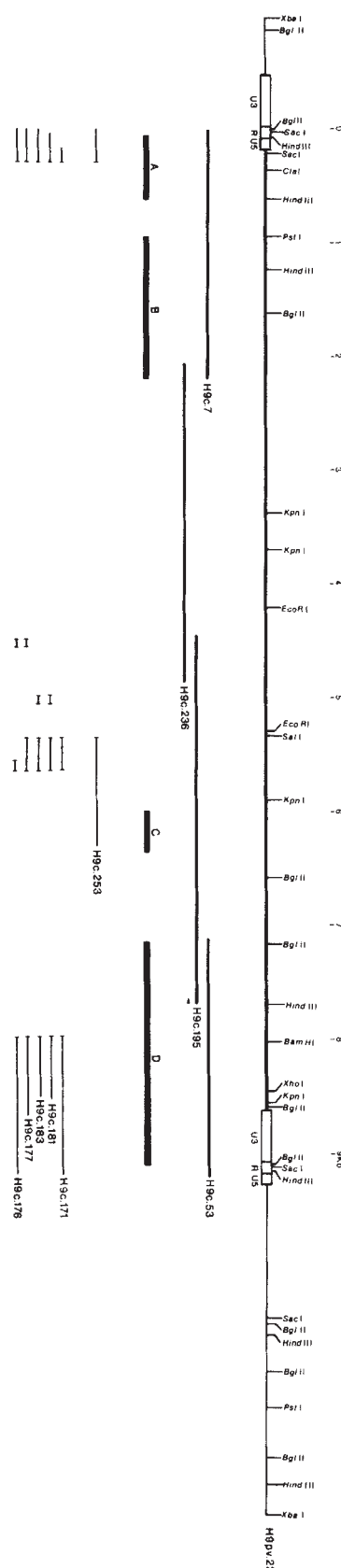
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Fig. 1 Map of LAV/HTLV-III proviral and cDNA clones. Proviral sequences (bold line), flanking cellular DNA (thin line), LTR regions (boxed areas) and restriction endonuclease sites are indicated at top. Sequences are numbered from the start of viral transcription. cDNAs representing the full-length viral RNA (shown immediately below the provirus) and spliced subgenomic RNAs (at bottom of figure) are shown with the respective clone designation to the right of its map (H9pv, proviral λ clone; H9c, viral cDNA clone). The horizontal bars labelled A, B, C and D refer to fragments used as probes for the identification of spliced cDNA clones. The arrowhead at nucleotides 7,687-7,702 denotes the 16-mer primer, 5'-CTCTGTCCCACTCCAT, used to prime cDNA synthesis of clone H9c.195.

Methods. Viral cDNA clones were isolated from a cDNA library prepared from H9/HTLV-III poly(A)⁺ RNA. Double-stranded cDNA was synthesized as described elsewhere⁵² and synthetic *EcoRI* adaptors were added⁵³. After the removal of excess adaptor, insertion into λ gt10⁵⁴ and *in vitro* packaging, the recombinant phage were plated at a density of $\sim 5 \times 10^5$ plaques per 150-mm plate. Replica nitrocellulose filters were made from the plates⁵⁵ and the library probed with the product of uniformly ³²P-labelled first-strand cDNA prepared as described above from poly(A)⁺ RNA from either H9/HTLV-III cells or the uninfected human T-cell line CEM⁵⁶. The filters were hybridized at 42 °C in a solution containing 5 $\times$ SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 $\times$ Denhardt's solution, 0.04 g l⁻¹ sonicated salmon sperm DNA, 50% formamide and 10% dextran sulphate and washed at the same temperature in 0.2 $\times$ SSC, 0.1% SDS. Plaques hybridizing specifically with the H9/HTLV-III probe were plaque purified and their DNA prepared for further analysis. To isolate clones comprising the remainder of the viral RNA genome, a synthetic oligodeoxynucleotide positioned near the 5' end of clone H9c.53 was used to prime specifically cDNA synthesis. One clone obtained from the resulting cDNA library, H9c.195, extended for 3.2 kb beyond the primer location but did not overlap into the region represented by H9c.7. Rescreening of the library with the H9c.195 insert allowed the recovery of a 3.5-kb clone, H9c.236, which covered the remaining viral sequences. To isolate clones containing the complete integrated provirus, *XbaI*-digested H9/HTLV-III genomic DNA was fractionated on sucrose gradients and the resulting 10-20-kb fragments were inserted into the *XbaI* cloning site of the bacteriophage λ vector J1 (ref. 57). Twenty-six clones containing an integrated provirus were recovered from $\sim 1 \times 10^6$ recombinants screened with clones H9c.7 and H9c.53.

with LAV infection, total DNA isolated from peripheral blood lymphocytes acutely infected with LAV was hybridized with H9c.7 and H9c.53 under stringent conditions. Fragments of similar sizes were detected in *HindIII*, *BglII* and *SstI* digests of DNA from H9/HTLV-III and LAV-infected cells (Fig. 2A). This result demonstrates clearly that HTLV-III and LAV correspond to the same or very closely related viruses. By contrast, H9c.7 and H9c.53 did not hybridize to cloned HTLV-I or HTLV-II provirus sequences, even in conditions of low stringency (data not shown). Significantly, the level of hybridization detected with DNA from LAV-infected lymphocytes was at least 20-fold greater than that with H9/HTLV-III DNA (Fig. 2A). Most of the hybridization observed with LAV-infected cell DNA migrates as a 9.5-kilobase (kb) species in *XbaI* digests (Fig. 2A, lane h) or with undigested DNA (data not shown), suggesting that this represented linear unintegrated viral DNA. By contrast, little unintegrated DNA was detected with H9/HTLV-III; instead, most of the DNA in the *XbaI* digest appeared in five or more discrete species of integrated proviruses 15-20 kb in size (Fig. 2A, lane g). Dot-blot hybridizations confirmed the presence of 5-10 copies of proviral DNA in H9/HTLV-III cells (data not shown). As no more than 5% of cells treated with LAV seemed to be infected by the virus by indirect immunofluorescence of viral antigens (data not shown), there thus seemed to be several hundred copies of unintegrated DNA present per LAV-infected cell.

Clones comprising the remainder of the viral RNA genome (H9c.236, H9c.195) were identified in a second cDNA library prepared with a specific primer (see Fig. 1). The regions represented by these four overlapping cDNA clones and their



restriction maps are shown in Fig. 1. The size predicted for the full-length viral RNA genome, 9.2 kb, is consistent with the largest species observed by blot analysis of H9/HTLV-III poly(A)⁺RNA (Fig. 2B). Given the possibility that the cDNA clones isolated might reflect RNA splicing events leading to the removal of small introns and therefore do not represent the entire viral genome, it was necessary to isolate molecular clones

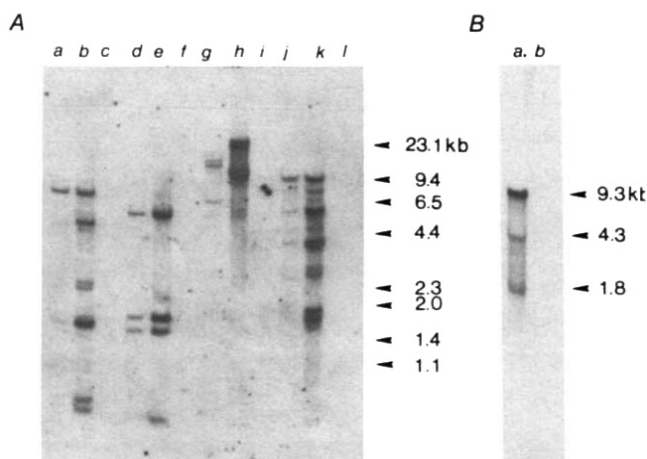


Fig. 2 Blot hybridization analysis of DNA and RNA from HTLV-III- and LAV-infected cells. **A**, Genomic DNA was isolated from the H9/HTLV-III cell line, acutely LAV-infected peripheral lymphocytes or normal blood leukocytes. Each (5 μ g) was digested with the indicated restriction endonuclease, electrophoresed on a 1% agarose gel and transferred to nitrocellulose⁵⁸. The blot was hybridized with ³²P-labelled probes⁵⁹ A, B and D (see Fig. 1) of equal specific activity using the hybridization and washing conditions described in Fig. 1 legend. The fragments obtained from *Hind*III digestion of λ DNA and *Hae*III digestion of Φ X174 were used as relative molecular mass markers; these sizes are shown in kilobase pairs (kb) on the right. Lanes *a*, *d*, *g*, *j*, HTLV-III-infected H9 DNA; lanes *b*, *e*, *h*, *k*, LAV-infected lymphocyte DNA; lanes *c*, *f*, *i*, *l*, normal leukocyte DNA. DNA in lanes *a*–*c* was digested with *Hind*III; *d*–*f*, *Bgl*II; *g*–*i*, *Xba*I; *j*–*l*, *Sst*I. **B**, 1 μ g poly(A)⁺ RNA from either HTLV-III-infected H9 cells (*a*), or non-infected H9 cells (*b*) was electrophoresed on a 1% formaldehyde/agarose gel⁶⁰, transferred to nitrocellulose and hybridized to ³²P-labelled probe D (see Fig. 1) in hybridization and washing conditions identical to those in Fig. 1 legend. The size of each predominant RNA species was estimated from migration of single-stranded DNA markers and is indicated on the right.

of the integrated provirus. A library of size-enriched DNA was constructed to isolate proviral clones taking advantage of absence of *Xba*I sites in the provirus apparent from both the Southern blot results (Fig. 2A) and the map of the cDNA clones (Fig. 1). The restriction map of the integrated provirus clone selected for sequence analysis, H9pv.22, showing the proviral and adjoining cellular sequences is presented in Fig. 1.

Nucleotide sequence analysis

The complete DNA sequence of the integrated provirus is presented in Fig. 3 and compared with that determined for the four overlapping cDNA clones. The co-linearity of these sequences confirms that the cDNA clones isolated represent the unspliced viral genomic RNA. A significant degree of nucleotide heterogeneity is displayed by the proviral and cDNA sequences. Similarly, nucleotide differences are evident in the overlap regions between cDNA clones. This genomic diversity probably reflects the observation that the H9/HTLV-III cell line studied here was established by infection with material from several AIDS patients¹⁰. Together with the blot hybridization data indicating the presence of about five intact provirus copies (Fig. 2A, lane *g*), these results suggest that two or more distinct virus isolates are integrated stably in the H9/HTLV-III cell line. Despite 68 nucleotide differences between the provirus and cDNA sequences, a consistent structure nevertheless emerges for the coding potential of the virus.

The long terminal repeats (LTRs) of the provirus exhibit the structural features common to all retroviral LTRs which reflect the manner of viral replication and are essential to the mode of entry of the virus into the host genome and recognition by the cellular transcriptional apparatus²⁵. The unique 5' (U5) and 3' (U3) regions are bordered by sequences complementary to the

Fig. 3 (Right) Complete nucleotide sequence of the HTLV-III/LAV provirus genome. The sequence shown represents the coding strand, comprising 9,213 bp extending from the RNA cap site to the polyadenylation site. The following features of the DNA sequence are indicated: the U5, R and U3 regions; splice acceptors (^{sa}) and splice donors (^{sd}); the inverted repeat located at the 5' end of U3 and the 3' end of U5 (IR); the tRNA^{Lys} primer binding site (PBS) and the (+)-strand initiation site (+). The polyadenylation signal (AATAAA) and Goldberg-Hogness sequence (TATAAG) are boxed. Deduced amino acid sequences of *gag*, *pol*, *P'*, *env*, and *E'* are shown above the DNA. The NH₂-terminus of p24^{gag} and that predicted for gp65^{env} and gp41^{env} (see text) are indicated. Nucleotide variations between cDNA and proviral isolates are below the line, and the resulting amino acid differences are above. The three nucleotides (TAA) indicated below the line at position 56–62 represent an insertion in the cDNA sequence.

Methods. cDNA inserts were mapped with restriction endonucleases, fragments isolated and cloned into M13 vectors⁶¹. Single-stranded template was isolated and the sequence determined using the chain termination method⁶². Additional fragments were sequenced to determine the overlap junctions. Using the completed cDNA sequence, overlapping fragments of ~800–1,000 nucleotides were isolated from the provirus clone H9pv.22 for comparative sequence analysis.

primers which copy the terminally redundant sequences (R) of the viral RNA to accomplish the series of intermolecular strand exchanges responsible for viral (–) and (+)-strand DNA synthesis. These borders correspond to the ends of the resulting linear duplex molecule from which, in all cases so far examined, two base pairs (bp) are lost on insertion of the provirus into cellular DNA²⁵. Based on this premise, the 5' end of U3 (nucleotide 8,662) and the 3' end of U5 (nucleotide 182) were identified from the sequence of the virus-cell DNA junctions in the H9pv.22 provirus clone (see Fig. 1). As predicted, a 23-bp sequence 3' to the U5 boundary is complementary to a potential primer for (–)-strand synthesis, transfer RNA^{Lys} the same primer used by the mouse mammary tumour virus (MMTV)²⁶, whereas that 5' to the boundary U3 consists of a stretch of 15 purines, the sequence found generally at the site of (+)-strand chain initiation²⁵.

In accord with the general retroviral paradigm, the integration event represented by the H9pv.22 clone reflects a duplication of host sequences at the insertion site; a short inverted repeat is found at the ends of the viral LTR. The virus-cell DNA junction sequence, TGTAGTGGGTG...CAGTGGGTGAT (viral sequences underlined), indicates a 5-bp duplication (GTGGG) of cellular DNA and an inverted repeat of 4 bp (ACTG...CAGT), two nucleotides of which are lost on insertion. In agreement with the general finding that the size of the duplication resulting from integration is a property of the virus and not the host cell, it is interesting that integration of HTLV-I, which shares OKT4 tropism with LAV/HTLV-III, results in a direct repeat of 6 bp of cellular DNA²⁷.

The 3' end of the viral RNA (Fig. 3) corresponds to the site of poly(A) addition in the H9c.53 cDNA clone and is 18 nucleotides from the polyadenylation signal, AATAAA²⁸. The 5' end of the viral RNA was mapped by *in vitro* extension of a synthetic DNA primer complementary to sequences in U5. As shown in Fig. 4, most of the RNA is initiated at the G residue indicated as position 1 (Fig. 3), although heterogeneity of two or three nucleotides is observed. Based on these results, the sizes of the U3, R and U5 regions are 456, 96 and 86 nucleotides, respectively. The RNA initiation site is located 23 nucleotides from the sequence TATAAG, which conforms to the consensus Goldberg-Hogness box found characteristically in this distance and implicated in the positioning of eukaryotic transcription initiation sites²⁹. A direct repeat of 10 bp is found 54 nucleotides 5' to this sequence (nucleotides 9,013–9,022; 9,027–9,036).

The *gag* gene encodes p24

The *gag* reading frame indicated by the provirus sequence extends from nucleotides 336–1,769 and, by analogy to other retroviruses, is expected to code for a precursor polypeptide

[illegible]



Fig. 4 Determination of the initiation site of viral transcription. Autoradiograph of a 5% polyacrylamide, 8 M urea gel. GATC, a DNA sequence ladder corresponding to the region surrounding the U3/R junction of the 5' LTR. Results of primer extension reactions are shown using as template poly(A)⁺ RNA prepared from HTLV-III-infected (a) or uninfected (b) H9 cells.

Methods. A synthetic 16-mer complementary to the sense (+)-strand in U5 (position 129–144, see Fig. 3) was annealed to a phage M13 single-stranded template containing a portion of the (+)-strand corresponding to U5, R and part of U3. The primer was extended by incubation at 37 °C for 30 min in the presence of [α -³²P]dCTP, [α -³²P]dATP, dTTP, dGTP and DNA polymerase I (Klenow fragment). The product was digested with *Hind*III, heated at 100 °C for 5 min and fractionated on a 6% polyacrylamide gel. The labelled, 63-base-long, single-stranded fragment extending from position 144 to the *Hind*III cleavage site at position 81 was recovered; $\sim 5 \times 10^5$ c.p.m. ³²P was annealed to 7.5 μ g poly(A)⁺ RNA in 80% formamide, 0.4 M NaCl, 40 mM PIPES (pH 6.5), 1 mM EDTA at 52 °C for 3 h. After primer extension with avian myeloblastosis virus reverse transcriptase, the sample was treated with 100 mM NaOH at 70 °C for 45 min and loaded onto a 5% polyacrylamide, 8 M urea gel. The DNA sequence used as marker was generated by using the above 16-mer as the primer in dideoxy method sequencing reactions on the same M13 template used to prepare the 63-bp fragment, therefore the primer extension products co-migrate with the corresponding nucleotide sequence.

which is post-translationally cleaved to give the internal structural proteins of the virus. Translation of this reading frame begins at the 5'-proximal ATG triplet of the viral genomic RNA and would lead to the synthesis of a 478 amino acid polypeptide consistent with the relative molecular mass (M_r) 53–55,000 of the gag-encoded polypeptide (Pr54^{gag}) detected recently in H9/HTLV-III cells³⁰. A good candidate for the major virion core protein encoded by this precursor is a 24,000 M_r virus-associated protein (p24) recognized by sera from AIDS and ARC patients^{7–9,15,16} (see Fig. 6). Recently, the N-terminal amino acid sequence of p24 has been determined (J. Bell and C.V.B., unpublished observations) and the 17-residue sequence

obtained for the purified protein matches exactly that beginning with the proline residue found at position 732 (Fig. 3).

The p24^{gag} N-terminal cleavage thus identified predicts a 132 amino acid protein from the N-terminus of the gag-encoded polypeptide, containing a single potential asparagine-linked N-glycosylation site. Interestingly, this cleavage recognition site (the aromatic amino acid proline) is analogous to that found in the precursor for Moloney murine leukaemia virus (Mo-MuLV)³¹, suggesting that processing occurs by a viral or cellular protease with similar specificity.

Although the proteolytic cleavage responsible for generating the C-terminus of p24^{gag} has not yet been defined, the presence of ~ 130 amino acids beyond the sequence sufficient to encode p24^{gag} indicates that a third protein is encoded by Pr54^{gag}. A direct repeat of 36 nucleotides resulting in a C-terminal duplication of 11 amino acids (positions 1,676–1,747) is obvious in this region. The sequence of this protein shows significant conservation of cysteine residues with p12^{gag} of Rous Sarcoma Virus (RSV), p11^{gag} of HTLV-I and p10^{gag} of Mo-MuLV^{27,31,32}. Common to each protein are three cysteine residues separated by two and nine amino acids, respectively; this structure is found twice in the HTLV-III, RSV and HTLV-I proteins and once in Mo-MuLV. The p12^{gag} of RSV, a protein rich in basic residues, is the major component of the virion ribonucleoprotein complex, binding nonspecifically to many sites on the viral RNA^{33,34}. Similarly, the HTLV-III protein is highly basic, containing 23 lysine and arginine residues. These striking similarities between otherwise highly-diverged proteins suggests that this C-terminal gag-encoded protein constitutes the core ribonucleoprotein.

The *pol* region

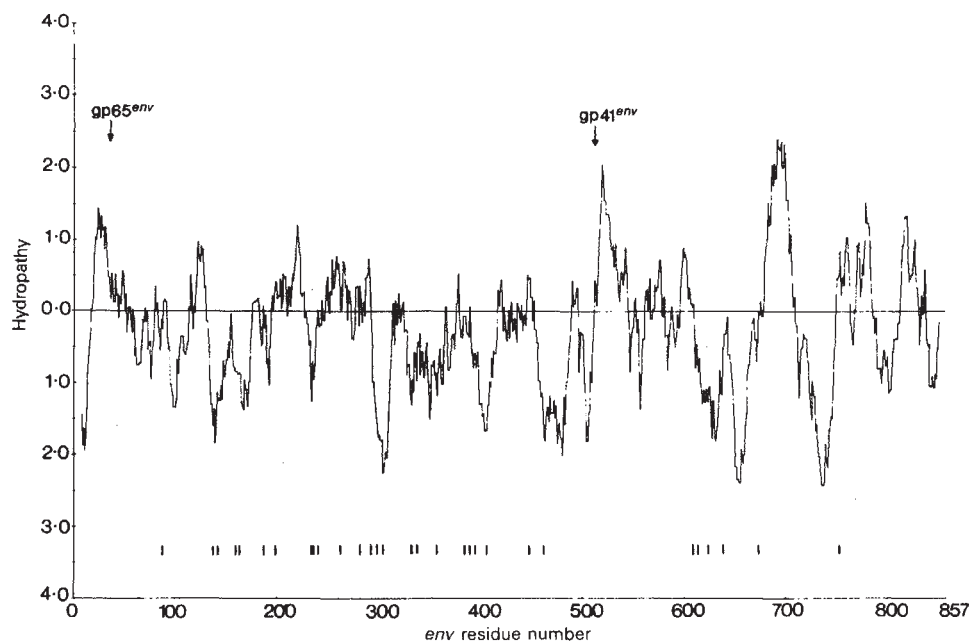
The product of the *pol* gene is encoded by the second large reading frame of the virus located between nucleotides 1,639–4,674. The predicted amino acid sequence shows significant homology to the *pol* gene products of RSV, HTLV-I, and Mo-MuLV (~ 20 –30%)^{27,31,32}, confirming the assignment of this reading frame to the *pol* gene. Despite the conservation of amino acid structure, however, there is no significant corresponding nucleotide homology with RSV, HTLV-I or Mo-MuLV, in contrast to earlier reports demonstrating cross-hybridization between HTLV-III and HTLV-I in this region^{35,36}. The reverse transcriptase of several avian and mammalian retroviruses is translated initially as a gag-pol-encoded polypeptide, an event for which either the suppression of an in-frame amber codon (Mo-MuLV) or the removal of a short interval by RNA splicing (RSV) has been suggested as a mechanism for joining the reading frames of the gag and pol genes^{31,32}. The gag and pol genes of HTLV-III are also situated in different reading frames (Fig. 3), suggesting a similar requirement for splicing as a mechanism of *pol* expression. The first ATG codon encountered in the *pol* gene is at position 1,939; however, the potential reading frame begins at position 1,639, overlapping the gag gene by 130 nucleotides. Thus, the possibility exists for shared amino acid sequences between the C-terminus of the gag-encoded ribonucleoprotein and the N-terminus of reverse transcriptase.

There exists a third reading frame, designated *P'*, between nucleotides 4,589–5,197, containing ATG triplets at positions 4,622, 4,643, 4,667 and 4,706 and could thus encode a protein of ~ 192 amino acids. The potential for generating spliced transcripts containing *P'* sequences suggests that this reading frame is used (see below). Beyond *P'* there is an intercistronic region of 602 nucleotides with frequent stop codons in all three reading frames. This contrasts with the compact organization of Mo-MuLV and HTLV-I that have overlapping *pol* and *env* genes^{27,31}.

The *env* gene

The primary translation product of the *env* gene of retroviruses is a large glycosylated precursor synthesized on the rough endoplasmic reticulum, which is processed to produce a larger N-terminal glycoprotein bearing the host range determinants and a smaller hydrophobic protein serving as the membrane anchor by virtue of a transmembrane domain. The *env* gene product

Fig. 5 Hydropathy plot for the *env* gene product. Hydrophobic areas appear above the midline, and hydrophilic areas below. Potential sites of asparagine-linked glycosylation are indicated by vertical lines below the plot. The NH₂-termini of the putative mature gp65^{env} and gp41^{env} are indicated. The region corresponding to gp65^{env} spans ~480 amino acids beginning after a hydrophobic leader and extending to the conserved processing site Arg-N-(Arg/Lys)-Arg which marks the NH₂-terminus of gp41^{env}. The gp41^{env} region consists of 345 amino acids containing two extended hydrophobic domains. Hydropathy was calculated by the method of Kyte and Doolittle⁶³ for each overlapping segment of 15 amino acids.



of HTLV-III is encoded by the large open reading frame at nucleotides 5,782–8,370. Analysis of a cDNA clone representing the *env* mRNA (see below) suggests that translation of the *env* region is initiated at the ATG codon located near the beginning of this reading frame at position 5,803. This assignment predicts the synthesis of an 856-residue envelope precursor protein containing 30 potential sites of asparagine glycosylation located principally in the first half of the molecule (Fig. 5). Taking into account the addition of carbohydrate, the predicted size of the *env* precursor approximates gp120, a 120,000 *M_r* glycoprotein detected recently in intracellularly labelled H9/HTLV-III cells³⁰.

Although N-terminal sequence information is presently unavailable for the envelope proteins of HTLV-III and there is no discernible amino acid homology with the *env* gene products of RSV, Mo-MuLV, HTLV-I or MMTV^{27,31,32,37}, certain features of the sequence allow the prediction of the processing of the precursor molecule. Three stretches of hydrophobic and non-polar residues are present at positions 5,851–5,886 (12 residues), 7,336–7,419 (28 residues) and 7,852–7,917 (22 residues) (Fig. 5). The first stretch is located at the N-terminus of the precursor and is flanked by charged residues, suggesting a role as the signal sequence responsible for directing the protein to the cell surface³⁸. The size and position of the other two hydrophobic regions suggest that they are located in the transmembrane protein of the HTLV-III envelope. The transmembrane envelope proteins of RSV, Mo-MuLV, MMTV and HTLV-I similarly display two hydrophobic stretches of 20–30 amino acids separated by ~150–200 residues^{27,31,32,37}. In each case, maturation of the envelope polyprotein involves cleavage after a conserved sequence of basic residues, Arg-N-(Arg/Lys)-Arg, immediately preceding the first hydrophobic stretch. The presence of this sequence at the corresponding position of the HTLV-III *env* gene product suggests that gp120^{env} is cleaved into a N-terminal protein of ~480 amino acids and a transmembrane protein of 345 amino acids with 24 and 6 potential asparagine-linked glycosylation sites, respectively (Fig. 5).

This assignment for the major envelope glycoprotein and transmembrane protein is supported by serological evidence. We analysed HTLV-III virion proteins by immunoblotting with antisera from individuals infected with the virus. With extensively diluted sera from infected individuals, we detect a predominant species of *M_r* 65,000 in addition to p24^{gag} (Fig. 6A–E), which does not react with undiluted sera from normal individuals (Fig. 6F) suggesting that p65, like p24^{gag}, is a major structural constituent of the virion. The size of p65 is consistent with the predicted size of the major envelope glycoprotein.

Previous studies have indicated the presence of a p65 in HTLV-III virion preparations and in H9/HTLV-III cells^{15,16,39}, but have consistently detected far greater amounts of a 41,000 *M_r* glycoprotein^{16,17} (also detected by the sera analysed in Fig. 6). In light of the present evidence, it seems that gp41 may represent the transmembrane protein rather than the major glycoprotein. The apparent difference in the ability of patient sera to detect p65 rather than gp41 in these studies may reflect the method of virus preparation.

Beyond the *env* gene there is a fifth open reading frame, designated *E'*, between nucleotides 8,347–8,992, extending into the U3 region of LTR. This novel reading frame can encode a protein of 206 amino acid residues, beginning at the ATG triplet at nucleotide 8,370, which is unrelated to the X genes of HTLV-I or HTLV-II (refs 27, 40, 41).

Transcription analysis

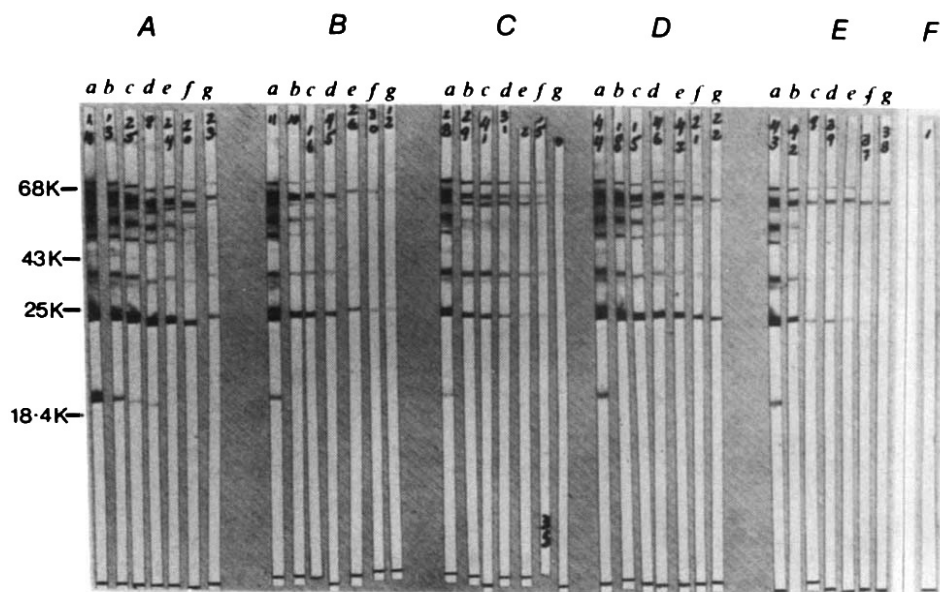
The synthesis of proteins encoded at internal sites in the retroviral genome is accomplished by two mechanisms; the post-translational cleavage of polyprotein precursors and for genes located in the 3' half of the genome (the *env* gene and transforming genes of several acute transforming viruses), by the expression of spliced subgenomic messenger RNAs. Northern analysis of H9/HTLV-III RNA revealed three prevalent viral specific RNAs of 9.3, 4.3 and 1.8 kb (Fig. 2B), suggesting the expression of three major virus-encoded primary translation products. To determine whether the 4.3- and 1.8-kb subgenomic RNAs could account for the synthesis of the HTLV-III *env* and *E'* gene products, viral transcription was analysed with hybridization probes specific for the detection of cDNA clones representing spliced transcripts. Evidence from several retroviruses indicates that sequences from the 5' end of the viral genome are found in each of the major viral RNAs^{42–44}. Therefore, we sought cDNA clones that would hybridize to probes derived from the 5' end of the virus (probe A) as well as the *env* (probes C, D) or *E'* regions (probe D), but not with sequences corresponding to the *gag-pol* region (probe B) (Fig. 1).

This screening strategy allowed the identification of two distinct classes of subgenomic mRNA clones. The structures of these transcripts (obtained by DNA sequence analysis) are presented in Fig. 1. The first class of spliced mRNAs, typified by clone H9c.253, is comparable to the RNA species encoding the envelope proteins of other retroviruses⁴⁵. Transcription of this class of mRNA is initiated in the 5' LTR, probably extends to the polyadenylation site in the 3' LTR and reflects the removal of a large intron between nucleotides 289–5,359 containing the

Fig. 6 Immunoblot detection of viral antigens. Virion-specific proteins were detected by sera from two healthy homosexual men (A, D), plasma from two other healthy homosexual men (B, C), serum from a sexual partner of an AIDS patient (E), but not by undiluted serum from a healthy control individual (F). Serum samples were undiluted (a), diluted 1:10 (b), 1:20 (c), 1:40 (d), 1:80 (e), 1:160 (f), 1:320 (g). At the highest sample dilutions, only p24 and p65 are detectable.

Methods. HTLV-III-infected H9 cells were grown in RPMI 1640 supplemented with 5% fetal bovine serum. Virus particles were concentrated from culture fluids by ultrafiltration and purified by banding on a 20–60% sucrose gradient in an SW 27 rotor centrifuged for 16 h at 27,000 r.p.m. Fractions between 30–40% were diluted and the viral particles pelleted by ultracentrifugation. Viral proteins were separated on a

12% SDS-polyacrylamide gel and transferred to nitrocellulose. The blot was cut into strips and incubated overnight at 25 °C with the patient sample. After washing, virus-specific protein bands were visualized by incubation with biotinylated goat anti-human IgG and subsequent incubation with avidin-horseradish peroxidase. M_r standards of 68, 43, 25 and 18.4 ($\times 10^3$) are shown.



gag, *pol* and *P'* genes. The size predicted for this RNA, 4,144 nucleotides plus poly(A), corresponds to the 4.3-kb species detected by Northern analysis (Fig. 2B). The putative *env* initiation codon is preceded by three ATG triplets at positions 5,412, 5,551 and 5,643; however, each is followed by a nearby in-frame stop codon located 216, 81 and 243 nucleotides 3', respectively. The selection of an ATG codon other than the most 5' proximal ATG triplet for translation initiation has been previously noted for *gag* and *env* expression in Mo-MuLV and RSV^{31,32}.

The second class of spliced mRNAs identified hybridized with probe D but not probe C, indicating that they did not contain most of the *env* gene. This class of mRNAs reflected a variety of splicing events, involving the removal of two or more introns (Fig. 1) with the generation of an RNA species similar in size (~1.8 kb) to the smaller subgenomic RNA seen by blot analysis (Fig. 2B). Each member of this class was formed from the 5' leader (exon 1) and sequences corresponding to the first 268 nucleotides of *env* leader (exon 4). In every case, the latter sequences were joined directly to the splice acceptor at position 7,957 in the C-terminal coding region of *env* (exon 7). In addition, several clones possessed an additional untranslated leader exon of 50 nucleotides (exon 2) (H9c.181, H9c.183) or 74 nucleotides (exon 3) (H9c.176, H9c.177) derived from the *pol* and *P'* regions, respectively. Table 1 summarizes the size and location of each exon and compares their donor and accep-

tor sequences. As a consequence of the splicing event which deletes most of the *env* gene, the ATG triplet at position 5,643 is removed whereas the distance between the ATG triplets at positions 5,412 and 5,551 and the next in-frame stop codons increases to 258 and 348 nucleotides, respectively. Neither of these reading frames connects with the sequences that encode the C-terminus of the *env* polyprotein. One of the clones (H9c.176) uses an alternative splice acceptor in exon 4, resulting in the juxtaposition of exon 2 with the last 69 nucleotides of the *env* leader (exon 5) and the removal of the ATG codons at 5,412 and 5,551. Significantly, the members of this diverse class of spliced RNAs are all related by their ability to direct the synthesis of the putative *E'* gene product.

Discussion

Although a definitive interpretation of the HTLV-III sequence awaits the demonstration of biological activity for our virus clones, the concordance of provirus and cDNA sequences corresponding to apparently distinct virus isolates suggests that they portray accurately the structural features of the viral genome. Despite a gene organization in many aspects similar to other retroviruses, the HTLV-III genome seems to be entirely unrelated by nucleotide homology to previously characterized retroviral sequences^{27,31,32,37}, including HTLV-I and HTLV-II which display a similar tropism for the OKT4⁺ T-cell subset⁴⁶. The

Table 1 Summary of LAV/HTLV-III exon and splice junctions

Exon	Location	Length (nucleotides)	Splice acceptor	Splice donor
1	1-289	289	—	... GACTG GTGAGTAC
2	4,494-4,543	50	TTTCGGGTTTATTACAG	GGA ... GAAAG GTGAAGGG
3	4,971-5,044	74	CTTTGACTGTTTTTTCAG	ACT ... ACAAG GTAGGATC
4	5,359-5,626	268	TGTTTATCCATTTTCAG	AAT ... AAGCA GTAAGTAG
5	5,558-5,626	69	GGCATCTCCTATGGCAG	GAA ... AAGCA GTAAGTAG
6	5,359-9,213	3,855	TGTTTATCCATTTTCAG	AAT ... —
7	7,957-9,213	1,257	CACCATTCGTTTCAG	ACC ... —

Each row lists the known exons (see Fig. 7), the nucleotide positions comprising each exon (see Fig. 3), and total exon length. The DNA sequence adjacent to the 5' (acceptor) and 3' (donor) borders at each exon are also shown. Intron sequences are to the left of the vertical line in the acceptor column, and to the right of the vertical line in the donor column. These sequences were obtained by comparison of the complete proviral sequence with those of several cloned cDNAs representing spliced subgenomic mRNAs, shown in Fig. 1.

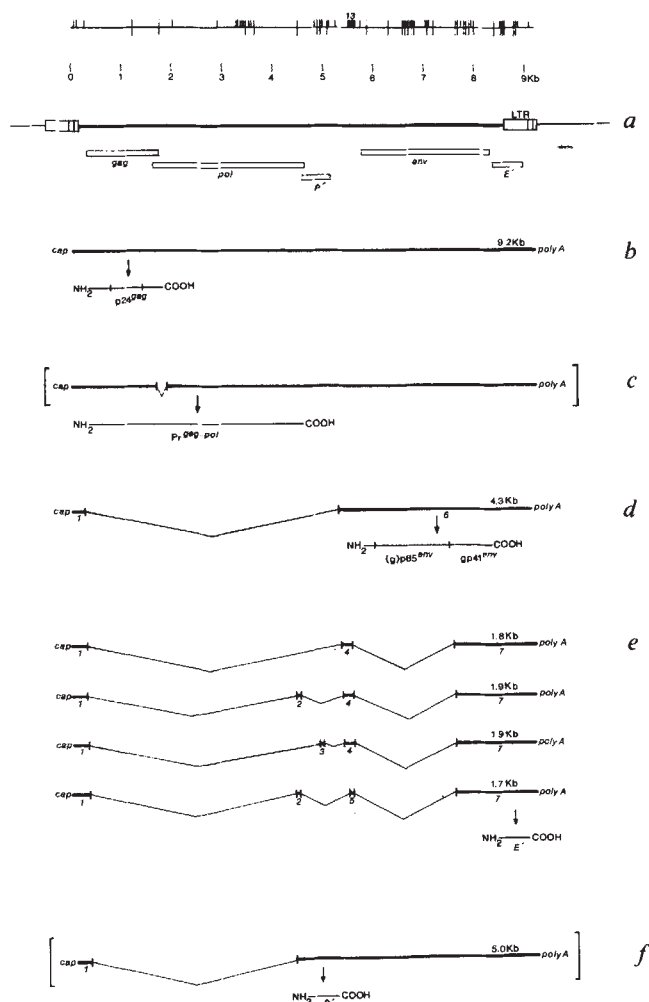


Fig. 7 Summary of viral gene organization and expression. *a*, Translational reading frames for the viral genes are indicated below a representation of the integrated proviral genome. Vertical marks above the line denote the location of nucleotide differences between the proviral (H9pv.22) and cDNA clones (H9c.7, H9c.236, H9c.195, H9c.53; vertical marks below the line indicate the resulting amino acid substitutions. *b*, Synthesis and subsequent processing of the *gag* precursor from full-length viral RNA. *c*, Hypothetical splicing pathway similar to that proposed for RSV³² which would allow for synthesis of a *gag-pol* fusion protein. *d*, Synthesis and processing of the *env* polyprotein from the 4.3-kb spliced, subgenomic mRNA. *e*, Predicted synthesis of *E'* gene product from the 1.8-kb class of spliced, subgenomic mRNAs. *f*, Hypothetical subgenomic RNA joining the 5' leader with exon 2 without subsequent splicing that could encode the predicted *P'* gene product.

most significant protein homology detected between the gene products of HTLV-III and other retroviruses is in the *pol* gene (~20–30%); however, a corresponding degree of nucleotide relatedness is not found, which is inconsistent with reports of homology to HTLV-I in the *gag-pol* region based on hybridization data^{35,36}. These results and the observation that the virus is morphologically distinct from the type C viruses^{8,13,15} lead us to propose that LAV/HTLV-III is a member of a novel class of retroviruses, perhaps including the equine infectious anaemia virus, which has a similar morphology and a serologically-related major core protein⁸.

Figure 7 summarizes the gene organization, transcriptional processing and polyprotein maturation pathways predicted for the virus. The *gag* gene product is a precursor of M_r 54,000 which, as found for other retroviruses, seems to be synthesized from genome-size viral mRNA (Fig. 7b). Pr54^{gag} is cleaved at two positions to generate the major core structural protein, p24^{gag}, a M_r ~15,000 N-terminal polypeptide and a structurally-

conserved C-terminal ribonucleoprotein of M_r ~15,000. The *pol* and *gag* genes are encoded by different reading frames; translation of a *gag-pol*-encoded polyprotein would thus require joining of the reading frames by a splicing event (Fig. 7c).

Characterization of a cDNA clone which probably represents the 4.3-kb major viral subgenomic RNA has allowed the identification of the transcript capable of directing the stoichiometric synthesis of the *env* gene products. The removal of a single intron containing the *gag-pol* region accomplishes the joining of a 289-nucleotide leader from the 5' end of the genome with a second untranslated leader situated within an intercistronic region between *pol* and *env* (Fig. 7d). Translation of this spliced subgenomic RNA would lead to synthesis of an 856 amino acid envelope polyprotein with a hydrophobic signal leader which would direct the precursor to the cell membrane and allow initiation of the viral envelope formation. Evidence of a conserved cleavage recognition site preceding an extended hydrophobic domain suggests processing of Pr120^{env} generates the major envelope and transmembrane glycoproteins. The sizes thus predicted for gp65^{env} and gp41^{env} are ~480 and 345 residues, respectively, in agreement with the observed electrophoretic mobilities of two major virion structural proteins. Demonstration of the glycoprotein nature of p65 or direct sequence analysis of these polypeptides will be required to affirm these assignments.

Expression of the novel *E'* gene product is indicated by a class of cDNA clones corresponding to several related but structurally heterogeneous spliced subgenomic RNAs of ~1.8 kb (Fig. 7e). The salient feature of this class is a tripartite structure consisting of the 5' leader, the intercistronic leader and 1.3 kb of RNA from the 3' end of the genome. Translation of the *E'* reading frame would result in the synthesis of a 206-residue protein lacking homology with the *X* gene products encoded by the 3' regions of HTLV-I and HTLV-II. Differentially-spliced mRNAs capable of encoding the *E'* gene product are generated by the addition of either of two additional untranslated leaders or by the use of an alternative splice acceptor in the intercistronic leader sequence. Although the existence of *E'* is unprecedented in other retroviral genomes, it is interesting to speculate that it acts as a virus-specific transcription factor, a function ascribed recently to the HTLV-I and HTLV-II *X* gene products^{47,48}. The multiplicity of splicing patterns also provides a rationale for the generation of a subgenomic mRNA directing the synthesis of the gene product encoded by the *P'* reading frame (Fig. 7f). In the examples already described, the joining of the 5' leader to the small exon in the *pol* gene (exon 2) is accompanied always by further splicing at the donor site located 50 nucleotides 3'. If further splicing at this donor did not occur, a subgenomic RNA ~870 nucleotides longer than the *env* mRNA would be produced having as its primary translation product a 192 amino acid protein specified by the *P'* reading frame. Alternatively, the removal of a small intron between the *pol* and *P'* reading frames could result in the synthesis of a *gag-pol-P'* precursor peptide. Further investigation will be required to establish the identity the *E'* and *P'* gene products and their roles in viral reproduction and pathogenesis.

Following retroviral infection, the major product of viral DNA synthesis in the cytoplasm is a linear, double-stranded molecule of genome size with a complete copy of the LTR at each end²⁵. Studies with several cytopathic retroviruses, notably spleen necrosis virus and the cytopathic subgroups of avian leukosis virus, have revealed a correlation between transient cell killing during acute infection and the transient accumulation of 100–200 copies of linear unintegrated viral DNA^{49–51}. Both transient cell killing and transient accumulation of linear unintegrated DNA require the spread of virus and superinfection of the infected cell population. Once a chronic state is established, the level of linear unintegrated DNA in the surviving cells decreases ~100-fold and is accompanied by the stable integration of several copies of the provirus. It is therefore interesting that similar preliminary observations have been made for LAV/HTLV-III infection of T lymphocytes. Southern blot analysis of DNA from

the chronically infected H9/HTLV-III T-cell line reveals 5–10 copies of integrated provirus, although there is little evidence for the persistence of more than a small amount of linear unintegrated DNA. By contrast, acute infection of human peripheral lymphocyte cultures with LAV leads to the apparent accumulation of >400 copies per cell of linear unintegrated DNA. Although H9/HTLV-III cells are relatively resistant to the cytopathic effects of HTLV-III¹⁰, the rapid disappearance of virus-producing cells in infected primary lymphocyte cultures suggests that LAV/HTLV-III has a severe cytopathic effect upon the natural host target cell (OKT4)^{8,11,14}. The level of linear unintegrated DNA in H9/HTLV-III cells and infected lymphocyte cultures thus seems to correlate with the relative cytopathic effects of the virus on these cells.

The H9/HTLV-III cell line was established by infection with material from several AIDS patients¹⁰ and therefore may contain sequences corresponding to different viral isolates. The distribution of nucleotide differences between the proviral and cDNA sequences described here must be interpreted with caution as we do not know the exact relationship of a particular clone to any of the multiple provirus copies present in the H9/HTLV-III cell line (Fig. 2A). Nevertheless, the independent origin of some of the clones is suggested by the large number of nucleotide

differences present (Fig. 7a). Particularly evident is a high degree of polymorphism in the *env* coding region (24 of 2,568 nucleotides are different) and the intergenic region (15/602). Eighteen of the changes in *env* lead to amino acid substitutions, mostly nonconservative. Two changes result in new potential sites of asparagine-linked glycosylation while one change removes such a site and therefore may be especially likely to have significant effects on protein antigenicity. Should the proclivity for change with the HTLV-III envelope seen here reflect a high rate of mutation *in vivo*, this may have significant implications for the ability of the immune system to respond effectively to the virus as well as for the design of vaccines.

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A *trans*-acting factor is responsible for the simian virus 40 enhancer activity *in vitro*

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Stimulation of in vitro transcription by the simian virus 40 enhancer involves a rapid and stable binding of a trans-acting factor with both the 5'- and 3'-domains of the enhancer sequence. The enhancer factor, which differs from other types of transcriptional factors, can interact with other enhancer elements.

REGULATION of gene expression at the level of transcription is probably an important control mechanism during development and in the terminally differentiated cells of eukaryotic organisms. This control may result from the interaction between specific DNA sequences, regulatory proteins and the transcrip-

tional machinery¹. The promoter DNA sequences involved in the control of transcription initiation in eukaryotic protein-coding genes are composed of several elements: the mRNA start-site, the TATA box sequence and one or several upstream elements, located generally in ~110 base pairs (bp) upstream

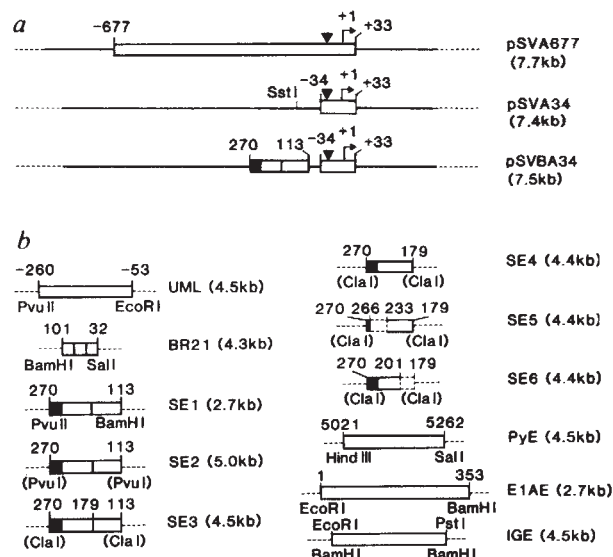


Fig. 1 Structure of the recombinants used as templates or as competitors in the *in vitro* transcription analysis. **a**, Hybrid constructions pSVA677, pSVA34 and pSVBA34 have been described previously^{19,20}. pSVA677 contains the entire Ad-2MLP between coordinates -677 and +33 with respect to the cap site. pSVA34 contains a deletion³⁰ which removes the upstream promoter sequences between -677 and -34 but leaves the TATA box (solid triangle) intact. pSVBA34 is derived from pSVA34 by inserting an SV40 fragment containing the 72-bp repeat (double open box, coordinates 113-251 of the BBB System described in ref. 28) and an additional 5'-flanking sequence (solid box) up to coordinate 270 into an *Sst*I site 63 bp upstream from the Ad-2MLP cap site²⁰. *Taq*I digests of these recombinants were used as templates in a HeLa whole-cell extract *in vitro* transcription system, generating a specific run-off of 525 nucleotides¹⁹. **b**, Structure of the plasmids used as competitors in the *in vitro* pre-binding transcription experiments. DNA fragments containing isolated enhancer sequences or upstream promoter elements were cloned into the pBR322 (ref. 31) sites as indicated. The plasmid UML contains the Ad-2MLP upstream sequences between positions -53 and -260 (ref. 20) inserted between the *Pvu*II and the *Eco*RI sites. BR21 contains the SV40 early promoter upstream element³² between coordinates 32 and 101 inserted between the *Sal*I and *Bam*HI sites. SE1 contains the SV40 enhancer from coordinates 113 to 270 (ref. 14) inserted between the *Bam*HI and *Pvu*II sites. SE2 and SE3 contain the same enhancer inserted in the *Pvu*I site and in the *Cla*I site, respectively (SE2 also contains an additional pBR322 0.5-kb insertion). SE4 is equivalent to SE3 but contains only a single 72-bp sequence (positions 179-270). SE5 is the same as SE4 but contains the deletion TB101 (refs 14, 19) in the 5'-domain of the enhancer element, between positions 233 and 266. SE6 is as SE4 but contains a deletion in the 3'-domain of the enhancer element, between positions 179 and 201. PyE is a plasmid containing the polyoma virus enhancer element (polyoma coordinates 5,021-5,262; ref. 22) inserted between the *Hind*III and *Sal*I sites. E1AE contains the Ad-2 E1A enhancer element (Ad-2 coordinates 1-353; refs 23, 24) inserted between the *Eco*RI and the *Bam*HI sites. IGE is a plasmid containing the mouse immunoglobulin heavy chain *Eco*RI-*Pst*I enhancer fragment isolated from MOPC11 cells⁹ inserted in the pBR322 *Bam*HI site. The coordinates and relevant restriction sites are indicated (restriction sites in parentheses were destroyed during cloning). The sizes of the plasmids are indicated in kb.

of the cap site^{2,3}. For some transcription units additional elements, enhancers, which have been found in simian virus 40 (SV40)^{4,5}, other viruses⁶ and recently in cellular genes⁷⁻⁹, are important for efficient transcription *in vivo*. Some enhancer elements have species-, tissue- or cell-specificity⁶⁻¹², suggesting an interaction with specific regulatory molecules and a possible role in the control of gene expression during differentiation. The SV40 enhancer, which contains the 72-bp repeat, has been studied extensively¹³⁻¹⁶ and has many characteristics in common with other enhancer sequences; it is a *cis*-acting bi-directional

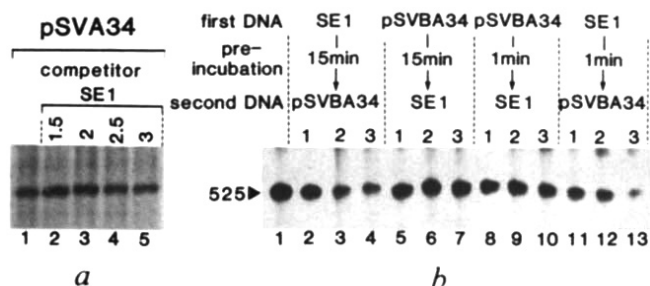


Fig. 2 A transcriptional factor interacts with the SV40 enhancer. **a**, *In vitro* transcription run-off analysis of the competition effect of SE1 on pSVA34 transcription: lane 1, transcription of the pSVA34 template alone; lanes 2-5, effect of preincubating the whole-cell extract for 15 min at 25°C with increasing molar amounts of SE1 competitor DNA (1.5-3 M excess of SE1 to pSVA34). **b**, Effect of SE1 competition on the *in vitro* transcription of pSVBA34: lane 1, transcription of the pSVBA34 template alone; lanes 2-4, whole-cell extract preincubated for 15 min with increasing amounts of SE1 (molar ratio to pSVBA34, 1-3) before addition of pSVBA34 template; lanes 5-7, preincubation of the whole-cell extract with pSVBA34 for 15 min and subsequent addition of the SE1 competitor DNA; lanes 8-10, as lanes 5-7 with a 1-min preincubation time; lanes 11-13, as lanes 2-4 with a 1-min preincubation time. The numbers above the electrophoretic gel lanes indicate the molar ratio of competitor to template DNA. The size of the run-off RNA is 525 nucleotides.

Methods. The *in vitro* transcription assay³³ and the preparation of HeLa whole-cell extract¹⁸ have been described previously. The DNA template for run-off assay was a complete *Taq*I digest with the amount of template present in each *in vitro* synthesis (also in Figs 3-6) being 250 ng per 25 µl reaction. The whole-cell extract (6-8 µl) in 18-20 µl buffer containing 10 mM Tris-HCl pH 7.9, 6 mM MgCl₂, 50 mM KCl, 5% glycerol, was preincubated for 15 min at 25°C with or without the appropriate competitor or template DNA, as indicated. The template and nucleoside triphosphates, pre-heated to 25°C, were added simultaneously. The amount of competitor DNA is expressed as a molar ratio with respect to the template. After addition of template, the *in vitro* synthesis was continued for 45 min. The RNA was then phenol- and chloroform-extracted, ethanol-precipitated and analysed on 5% acrylamide/8.3 M urea gels³⁴.

potentiator of initiation from homologous or heterologous promoter elements. Although it activates preferentially proximal promoter elements, it can act over considerable distances (several kilobases, kb), albeit with a decreased efficiency. Furthermore, DNase I hypersensitivity assays and electron microscopy have shown that the SV40 enhancer induces an alteration in chromatin structure¹⁷. Recently, with crude whole-cell extracts¹⁸ that have been used extensively to analyse the promoter DNA sequence requirements for RNA polymerase B (II) transcription, we have demonstrated that the SV40 enhancer can stimulate specific *in vitro* transcription from heterologous promoter elements¹⁹. Moreover, deletion mutations that decrease strongly enhancer activity *in vivo* also abolished the *in vitro* stimulation, suggesting that the *in vitro* stimulation reflects the enhancer activity *in vivo* (see ref. 19). As the template does not seem to be organized into a chromatin structure during *in vitro* synthesis^{19,20}, this result implies that the observed stimulation was due to the interaction between some transcriptional factor(s) and the enhancer sequence.

We report here results from *in vitro* competition experiments which indicate that a *trans*-acting factor(s) is responsible for the SV40 enhancer activity *in vitro*. We show also that the enhancer factor is different from those which interact with the upstream elements of the adenovirus-2 major late (Ad-2ML) or the SV40 early (21-bp repeat region) promoters. It appears that a stable complex is formed between the enhancer and the *trans*-acting factor(s) and that sequences in both the 3'- and 5'-regions of the 72-bp repeat are involved in the formation of this complex. Finally, we analyse the ability of other enhancer

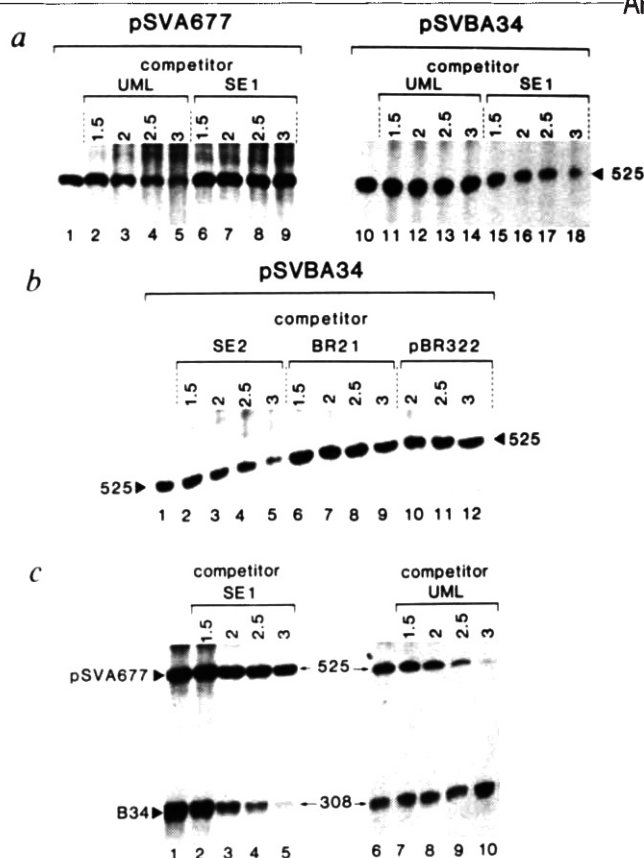


Fig. 3 The factors interacting with SV40 and Ad-2ML upstream promoter elements and enhancer are different. **a**, Run-off analysis of the effect of competition by UML and SE1 on transcription from pSVA677 (lanes 1–9) and pSVBA34 (lanes 10–18): lane 1, pSVA677 template transcription in the absence of competitor DNA; lanes 2–5, competition effect of UML as its concentration is increased in the preincubation (from 1.5 to 3 M ratio); lanes 6–9, effect of preincubating SE1 at increasing molar ratios; lane 10, RNA synthesized with pSVBA34 template in the absence of competitor DNA; lanes 11–14, absence of competition effect by UML at molar ratios increasing from 1.5 to 3; lanes 15–18, increasing competition effect of SE1 on pSVBA34 transcription using the same range of molar ratios. **b**, Run-off analysis of the effects of SE2, BR21 or pBR322 competitor DNA on pSVBA34 transcription: lane 1, pSVBA34 transcription in the absence of competitor; lanes 2–5, competition when SE2 is preincubated with the whole-cell extract at molar ratios increasing from 1.5 to 3; lanes 6–9, increasing amounts of BR21 used for the preincubation; lanes 10–12, effect of preincubating the whole-cell extract with pBR322 DNA before addition of the pSVBA34 template. **c**, Run-off analysis of the effect of competition by SE1 (lanes 1–5) and UML (lanes 6–10) on transcription from pSVA677 and B34 (B34 is described in Fig. 4 legend) when mixed in the same transcription reaction: lane 1, pSVA677 and B34 transcription in the absence of competitor (the total DNA amount in the reaction was 300 ng; 200 ng B34 and 100 ng pSVA677); lanes 2–5, competition when SE1 was preincubated with the whole-cell extract at increasing molar ratios; lane 6, pSVA677 and B34 transcription in the absence of competitor (the total DNA amount in the reaction was 200 ng; 140 ng B34 and 60 ng pSVA677); lanes 7–10, competition when UML was preincubated with the whole-cell extract at increasing molar ratios. The numbers above the electrophoretic gel lanes indicate the molar ratio of competitor to template RNA. The size of the run-off RNA was 525 nucleotides. Methods as in Fig. 2 legend.

sequences (polyoma virus, adenovirus 2 (Ad-2) and mouse immunoglobulin heavy chain) to compete with the *in vitro* enhancer activity of the SV40 72-bp repeat.

A *trans*-acting enhancer factor

We have reported previously that, using a HeLa whole-cell extract, the SV40 enhancer stimulates *in cis* transcription *in vitro* from heterologous promoter elements irrespective of its orientation. Deletions which diminish the enhancer function *in vivo* also decreased its activity *in vitro*. Inserting the SV40 72-bp repeat in close apposition to the –34 to +33 Ad-2MLP promoter (Ad-2MLP) element gave a 10-fold stimulation of specific *in vitro* transcription (ref. 19; compare A34 with B34 in Fig. 4c). To determine whether this *in vitro* stimulation is due to a *trans*-acting factor(s), we used an *in vitro* competition assay in which HeLa whole-cell extract was preincubated with increasing amounts of competitor DNA containing isolated promoter or enhancer elements for 15 min at 25 °C, then template and nucleoside triphosphates were added. Preincubation of the whole-cell extract with increasing molar amounts of SE1 (a pBR322 plasmid containing only the SV40 enhancer, Fig. 1) decreased specific transcription from the –34 to +33 Ad-2MLP element only when it was linked to the SV40 enhancer (compare pSVBA34 and pSVA34 templates in Fig. 2a, lanes 1–5 and Fig. 2b, lanes 1–4; see also Fig. 1). We find that the amount of transcription from the competed pSVBA34 was similar to that from pSVA34, indicating that stimulation of *in vitro* transcription by the enhancer involves a *trans*-acting factor(s).

The stability of interaction of the enhancer factor with the SV40 72-bp repeat was analysed in a pre-binding experiment in which the pSVBA34 template, instead of the SE1 plasmid, was preincubated with the whole-cell extract (Fig. 2b, lanes 5–7). The second DNA (in this case SE1 instead of pSVBA34) was added in increasing molar amounts after 15 min. No competition was observed, even when a three-fold molar excess of SE1 competitor DNA was added. When the time of pre-binding was reduced to 1 min (Fig. 2b, lanes 8–13), no competition was visible when pSVBA34 was preincubated (lanes 8–10), whereas competition did occur when the SE1 competitor DNA was

present during preincubation (lanes 11–13). We conclude, therefore, that *in vitro* stimulation by the SV40 enhancer involves the rapid formation of a stable enhancer-factor complex. That transcription from the pSVA34 template was not inhibited by preincubation with the enhancer-containing plasmid SE1 also suggests strongly that the factor which interacts with the enhancer is not required for transcription from a template containing only the TATA box region.

Specificity of the enhancer factor

To investigate whether the enhancer factor is different from factors which interact with upstream promoter elements, preincubations were carried out with plasmids containing either the SV40 early or the Ad-2MLP promoter upstream elements. It has been shown that the Sp1 factor²¹ which binds to the 21-bp repeat is different from that interacting with the upstream element of the Ad-2MLP (refs 21, 36). Specific transcription from pSVA677 (see Fig. 1), a plasmid containing the intact Ad-2MLP¹⁹, was decreased by preincubation with recombinant plasmid UML containing the Ad-2MLP upstream sequences between –53 and –260 (Fig. 3a, lanes 2–5), but not by preincubation with SE1, even at the highest template/competitor molar ratios (lanes 6–9). The converse experiment, using pSVBA34 as template, gave the expected results. Transcription from pSVBA34 was inhibited by preincubation with SE1 (lanes 15–18), but not with UML (lanes 11–14). The same results were obtained when pSVA677 and B34 (a template equivalent to pSVBA34 but generating a 308-nucleotide run-off transcript; see Fig. 4 legend) were mixed together and incubated in the same reaction assay (Fig. 3c). Preincubation with increasing amounts of SE1 decreased selectively transcription from the enhancer-containing template B34 (lanes 2–5), whereas preincubation with UML reduced transcription from pSVA677, but not from B34 (lanes 7–10).

These results indicate that the factor interacting with the upstream region of the Ad-2MLP is different from that interacting with the SV40 enhancer and also that the *in vitro* stimulatory effect of the SV40 enhancer, using the Ad-2MLP hybrid constructions (see ref. 19 and Fig. 2), is not due to the interaction

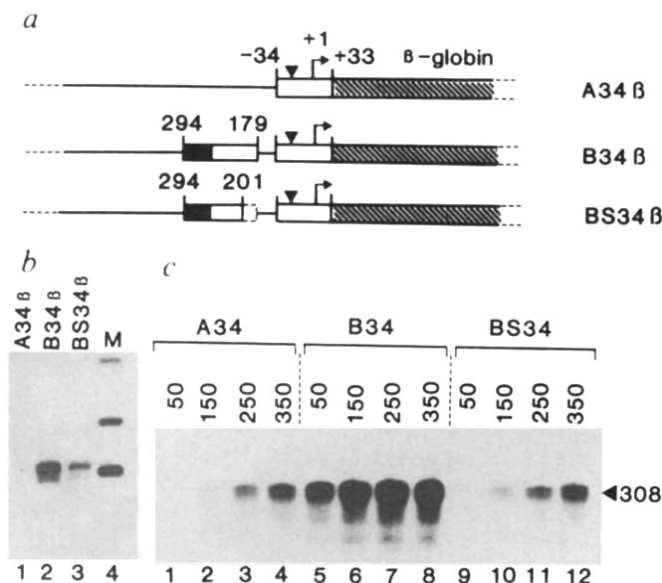


Fig. 4 Effect of deletion of the 3'-domain of the SV40 enhancer on the transcription from the -34/+33 Ad-2MLP element. **a**, Structure of recombinants A34β, B34β and BS34β. A34β was constructed as follows: the *Eco*RI-*Bam*HI fragment of pSVA34 (ref. 20) containing the -34/+33 Ad-2MLP element was inserted between the *Eco*RI and *Bam*HI sites of pBR322; the *Pvu*II fragment of the rabbit β-globin gene³⁵ (-9/+1650) was then blunt-end inserted into the *Bam*HI site. B34β is as A34β, but the SV40 enhancer-containing fragment (coordinates 179-294) is inserted in the *Sst*I site located at -63 with respect to the Ad-2MLP cap site (ref. 20; see also Fig. 1). BS34β is as B34β but the 3'-domain of the SV40 enhancer element is deleted between coordinates 201 and 179. **b**, S₁ nuclease mapping analysis of *in vivo* RNA transcripts after calcium phosphate transfection of HeLa cells with A34β, B34β and BS34β as indicated: lane M, size markers (³²P-end-labelled *Msp*I fragments of pBR322 are 180, 160 and 147 nucleotides in long; ref. 31). **c**, Run-off analysis of the RNA synthesized *in vitro*. The templates used are the same as in the *in vivo* analysis but lack the β-globin coding sequence, thus are called A34, B34 and BS34. Templates were linearized at the pBR322 *Sal*I site and the specific run-off RNA is 308 nucleotides long. Increasing amounts of template DNA (A34, B34 and BS34), varying from 50 to 350 ng per 25 μl reaction, were used as indicated.

Methods. Calcium-phosphate transfection into HeLa cells was carried out as described previously²⁰. We transfected 20 μg of each recombinant per 10-cm Petri dish and after 48 h, cytoplasmic RNA was isolated from cells lysed with 0.3% Nonidet P-40. For S₁ nuclease-mapping, RNA aliquots were dissolved in 10 μl PIPES, pH 6.5, 0.4 M NaCl containing an excess of single-stranded rabbit β-globin probe (position +138 to -84) 5'-labelled at the *Bst*NI site³⁵. The hybridization was carried out at 68 °C for 12 h and after digestion with S₁ nuclease, the DNA fragments were analysed on an 8% acrylamide/8.3 M urea sequencing gel³⁴.

between the 72-bp repeat and a factor(s) that binds to the Ad-2ML upstream promoter element.

To investigate whether the SV40 early upstream promoter region can bind the enhancer factor, competition experiments were performed using pSVBA34 as template and a pBR322-derived plasmid containing the 21-bp repeat sequence as competitor (BR21; see Fig. 1). Figure 3b, lanes 2-5 show the expected competition with the enhancer-containing plasmid SE2 (similar to SE1, but with the SV40 enhancer inserted elsewhere in pBR322; see Fig. 1). Neither the 21-bp repeat element (plasmid BR21, lanes 6-9) nor pBR322 alone (lanes 10-12) could compete for transcription from pSVBA34. Thus, the SV40 Sp1 factor does not seem to be responsible for the *in vitro* stimulation brought about by the SV40 enhancer.

Enhancer domains in factor binding

Stimulation of *in vivo* and *in vitro* transcription by the SV40 enhancer containing a single 72-bp sequence is decreased drasti-

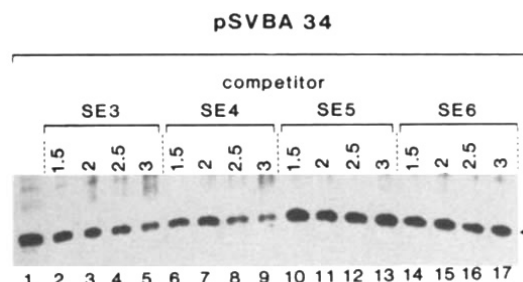


Fig. 5 Levels of competition by SV40 enhancer containing either a single 72-bp sequence (SE4) or the 72-bp repeat (SE3) are similar, whereas competitors containing internal deletions in the enhancer element are less efficient (SE5 and SE6; see Fig. 1). The template was pSVBA34 and the position of the specific run-off RNA (525 nucleotides long) is indicated by an arrowhead: lane 1, pSVBA34 transcription without competitor DNA; lanes 2-5, competition activity of SE3 preincubated with the whole-cell extract at molar ratios increasing from 1.5 to 3; lanes 6-9, competition by SE4; lanes 10-13, lack of competition by SE5; lanes 14-17, lack of competition by SE6. The numbers above the electrophoretic gel lanes indicate the molar ratio of competitor to template DNA. Methods as in Fig. 2 legend.

cally by a deletion which removes the 5'-domain of this sequence (TB101 in refs 14, 19, 20 and present in SE5; Fig. 1b). A deletion in the 3'-domain of an enhancer containing a single 72-bp sequence (between positions 179 and 201; see SE6 in Fig. 1b), results in a 15-fold decrease of *in vivo* transcription from the SV40 early promoter (M. Zenke, T. Grundström, H. Matthes, M. Wintzerith and P.C., in preparation). A similar decrease was observed *in vivo* using heterologous constructions (Fig. 4a) in which transcription from the Ad-2MLP was potentiated by the SV40 enhancer (compare lanes 1-3 in Fig. 4b). The effect of this 3'-domain deletion on Ad-2MLP activity was investigated *in vitro* (Fig. 4c). With comparable DNA concentrations, transcription of the template containing the 3'-domain deletion (BS34, lanes 9-12) decreased to a level similar to that observed with an enhancer-less template (A34, lanes 1-4).

Competition experiments were performed to determine whether the two domains important for enhancer function are required for factor(s) binding. Four analogous enhancer constructions (Fig. 1b) containing either the 72-bp repeat (SE3), a single 72-bp sequence (SE4), an enhancer carrying the 5'-domain deletion (SE5) or an enhancer carrying a 3'-domain deletion (SE6), were used as competitors. Using pSVBA34 as a template, SE3 and SE4 gave similar competitions (Fig. 5, lanes 2-9) analogous to those obtained with other recombinants containing the 72-bp repeat inserted in a different sequence environment (SE1 and SE2; see Figs 2, 3). In contrast, no significant competition was observed with SE5 and SE6 at the same DNA competitor concentrations (lanes 10-17). At higher SE5 and SE6 competitor/template molar ratios (that is, four and five), however, a partial competition was observed (data not shown). We conclude that both 5'- and 3'-domains of the SV40 enhancer are required for full enhancer activity and efficient binding of the enhancer factor(s).

Competition with other enhancer elements

To determine whether other enhancer elements could compete the stimulatory activity of the 72-bp repeat *in vitro*, we constructed recombinants containing the polyoma virus²², Ad-2 E1A^{23,24} or mouse immunoglobulin heavy chain⁷⁻⁹ enhancer sequences (PyE, E1AE and IGE in Fig. 1). These recombinants were tested in an *in vitro* competition assay, using pSVBA34 or pSVA677 as templates (Figs 6, 7c). Transcription from pSVBA34 was decreased when the whole-cell extract was preincubated with the Ad-2 E1A or immunoglobulin enhancer competitors (E1AE and IGE, lanes 6-9 and 10-13 in Fig. 6a), whereas the polyoma enhancer competitor (PyE in lanes 2-5 of Fig. 6a) affected only moderately pSVBA34 transcription. Transcription of pSVA677, which was not decreased by preincubation with

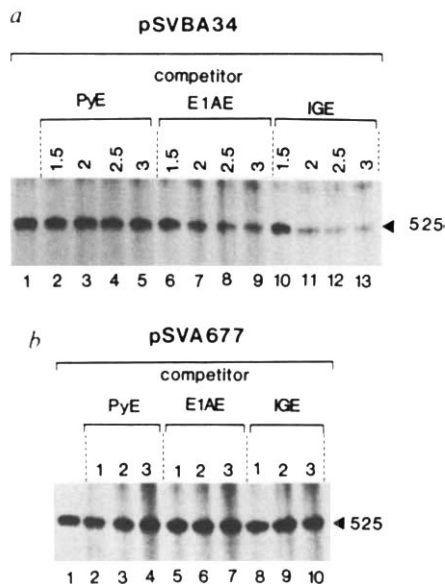


Fig. 6 Competition activity of various enhancer elements. **a**, Run-off analysis of pSVBA34 transcription competed by plasmids containing polyoma virus (PyE)²², Ad-2 E1A (E1AE)^{23,24}, or mouse immunoglobulin heavy chain (IGE)⁹ enhancers: lane 1, transcription of pSVBA34 alone; lanes 2–13, effect of preincubation with PyE, E1AE and IGE, as indicated. **b**, Run-off analysis of pSVA677 transcription competed by enhancer-containing plasmids PyE, E1AE and IGE: lane 1, transcription of pSVA677 alone; lanes 2–10, effect of preincubation with PyE, E1AE and IGE, as indicated. Molar ratios of competitor to template DNA are indicated above the electrophoretic gel lanes. Methods as in Fig. 2 legend.

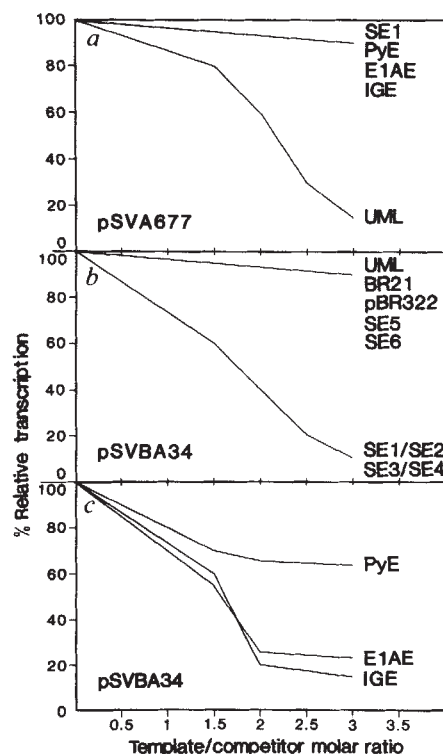


Fig. 7 Activity of the different competitor DNAs on *in vitro* transcription of pSVA677 and pSVBA34 templates summarizing the results from several experiments similar to those shown in Figs 2, 3, 5 and 6. **a**, Transcription of the enhancer-less Ad-2MLP template pSVA677 is decreased by preincubation of the whole-cell extract with UML which contains the Ad-2MLP upstream element (see Fig. 1), whereas SE1, PyE, E1AE and IGE competitors have no effect. **b**, Transcription of the enhancer-containing pSVBA34 template is decreased by preincubation of the whole-cell extract with competitors containing the SV40 enhancer with either an intact 72-bp repeat (SE1, SE2, SE3) or a single 72-bp sequence (SE4), whereas other competitors (UML, BR21, SE5, SE6 or pBR322; see Fig. 1) have no effect. **c**, Transcription of pSVBA34 is decreased to different extents by preincubation of the whole-cell extract with polyoma virus, Ad-2 E1A or immunoglobulin heavy-chain enhancer competitors (PyE, E1A and IGE, respectively; see Fig. 1). Each competition experiment was repeated at least three times; for each experiment quantitation was achieved by densitometer scanning of several autoradiograms corresponding to different exposure times. The relative transcription of a template is plotted as a function of increasing competitor/template molar ratios and expressed as a percentage of its transcriptional level in the absence of competition.

an SV40 enhancer competitor (SE1 in Fig. 3a), also was not affected by preincubation with the other enhancer competitors (Fig. 6b).

Conclusions

We have proposed previously that both generation of an altered chromatin structure and binding of transcription factor(s) could be part of the mechanism for transcription activation enhancer elements^{14,17,19}. That specific factor(s) may interact with enhancer elements has been deduced also from *in vivo* studies showing that various viral and cellular enhancers have different species- and/or cell-specificity^{6–12} and that the activity of a given enhancer on a promoter element could be competed *in vivo* by simultaneous transfection of a recombinant containing the enhancer element²⁵. Our *in vitro* results, quantitatively summarized in Fig. 7, support strongly the hypothesis that binding of a transcription factor(s) is involved in enhancer activity. This factor(s) is clearly different from those factors which stimulate transcription by binding to the TATA box^{26,27} or the upstream elements of the Ad-2ML and SV40 early promoters^{20,21,36} (see Figs 3, 7a, b). Furthermore, in the present *in vitro* conditions, the enhancer sequence and the factor(s) form a stable complex. The same 5' and 3' domains critical for enhancer activity *in vivo* are required for *in vitro* formation of this complex (see Figs 2, 4, 5 and 7b), implying either a relatively large protein contact site, the binding of more than one protein or a coiled enhancer sequence. Both the activity of the enhancer *in vitro* and its ability to form the stable complex are observed with a linear template and thus do not require superhelicity.

As the polyoma virus and SV40 are related²⁸, it is surprising that the polyoma enhancer competes only moderately for the SV40 enhancer factor(s), whereas efficient competition was observed with the mouse heavy chain immunoglobulin and Ad-2 enhancer elements (see Figs 6, 7c). Sequence comparisons of these enhancers indicate that all three contain the so-called core sequence^{24,29}. Clearly, this sequence is not sufficient to bind stably the SV40 enhancer factor(s), in agreement with the

requirement of both 5' and 3' domains of the SV40 enhancer for full activity. Thus, it is probable that the E1A and immunoglobulin enhancers have additional sequence features in common with the SV40 enhancer that are not present in the polyoma virus enhancer. We did not expect the immunoglobulin enhancer to compete as well as the E1A enhancer, as in a HeLa whole-cell extract system the latter stimulates transcription (P.S.-C., unpublished observation), whereas the immunoglobulin enhancer sequences used here do not (J. Dougherty and P. Augereau, personal communication). These seemingly contradictory findings may be explained by the observation (J. Dougherty and P. Augereau; X. Sen and D. Baltimore, personal communications) that the present immunoglobulin enhancer fragment also contains a sequence which mediates a transcriptional 'repression' in a HeLa whole-cell extract. Such a sequence could mask the stimulatory activity of the enhancer element present in the same fragment, but could not interfere with its ability to bind the SV40 enhancer factor(s).

Purification studies using the present enhancer-specific *in vitro*

competition assay will enable us to determine whether more than one factor binds to the SV40 enhancer and to investigate how the enhancer factor(s) interacts with RNA polymerase and transcription factors specific for the other promoter elements and whether it is involved in the generation of the open chromatin structure.

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LETTERS TO NATURE

The alignment of distant radio sources

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The relative orientation of the axes of extragalactic extended radio sources was first investigated by Willson¹, who noted that in the 3CR sample there was a tendency for the axes to lie parallel to each other for sources separated by $\leq 10^\circ$ on the sky. Using two larger samples, Sanders² has recently concluded that this tendency to align is present at a high confidence level ($>3\sigma$) only for distant (redshift, $z > 0.4$) sources and could arise from gravitational lensing by superclusters, if most of the matter in a closed universe is in thin filaments^{2,3}. Because of the important cosmological implications, we have now attempted to verify the effect using two more independent and larger source samples and find no significant tendency for axis alignment. In view of this negative result, we also investigated a sample formed by adding well-observed radio quasars to 3CR sources (similar to one of the two samples used by Sanders) and found that, even in this sample, the effect shows up only at a marginally significant level ($<2\sigma$).

Our first sample is drawn from a list of ~ 800 sources for which at least two lunar occultation scans along different position angles have been observed with the Ooty radio telescope. These observations have provided both accurate positions and brightness distributions with resolutions of $\sim 1-10$ arc s for sources with a flux density ≥ 0.25 Jy at 327 MHz. From the basic lists of Ooty occultation courses (ref. 4 and references therein; A.K.S., in preparation), we have formed a subsample of all 203 sources having a clear double or triple structure and for which

the radio axes have been determined unambiguously. In only a few cases is the uncertainty in the position angle (PA) likely to exceed $\sim 10^\circ$. The sources lie in a narrow band in the sky (declination width, $\sim 3^\circ-10^\circ$) covered by the Moon's path between 1970 and 1979. Although the sample is not strictly complete to a limiting flux density, it is expected to be unbiased as far as the PAs of the radio axes are concerned, which are, in fact, uniformly distributed between -90° and $+90^\circ$.

After Willson¹ and Sanders², we have estimated for each pair of sources in the sample both the angular distance between them, D , measured along a great circle and the angle X (reduced to the interval $0-90^\circ$) between the axes of the two sources measured with respect to the connecting great circle. For the total sample (203 sources), the resulting distribution of $\bar{X}$ (average value of X) for all pairs with values of D in 5° bins (Fig. 1a) shows no significant departure from the value $\bar{X} = 45^\circ$ expected if the radio axes are randomly oriented.

The accurate radio positions obtained from occultations have resulted in reliable optical identifications from the prints of the Palomar Sky Survey. At the flux level of the sample, only $\sim 30\%$ of the sources are identified; the remainder are expected to be quite distant, with $z \geq 0.4$. Thus, to obtain a more restricted sample, with $z > 0.4$, we omit sources identified with galaxies of $m_{\text{pg}} < 20$ and those at a galactic latitude $|b| < 10^\circ$, but those identified with quasars or stellar objects of unknown redshift are retained because most should have $z > 0.4$. The 'distant' sample of 141 sources (Fig. 1b) again shows no significant departure from $\bar{X} = 45^\circ$, with $\bar{X}(D < 5^\circ) = 45.1^\circ$ for 254 pairs. Because of the large number of pairs with $D < 5^\circ$, we have also used smaller intervals of D ; for the 84 pairs with $D < 2.5^\circ$, we find $\bar{X} = 47.4^\circ$.

Our second sample is a complete sample selected from the GB and GB2 radio surveys at 1,400 MHz, with threshold flux $S_{1400} \geq 0.55$ Jy (ref. 5), covering two different strips in declination, roughly between RA 7 and 17 h, which are $6-8^\circ$ wide and are centred at declination $\delta = +48.5^\circ$ and $+36^\circ$ respectively. The sample consists of 237 sources (83 and 154 respectively, in the two regions), most of which have been mapped^{6,7} using the Very

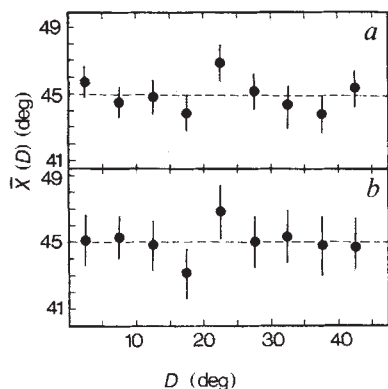


Fig. 1 The mean angle ($\bar{X}$) between the axes of radio sources as a function of the angular separation between them, D , for the Ooty occultation sample; *a*, for all 203 sources; *b*, for the 141 sources estimated to have $z > 0.4$. The error bars on each point are $\pm 45^\circ/\sqrt{3n_p}$, n_p being the number of pairs involved.

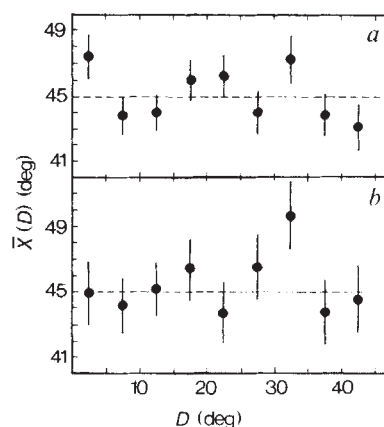


Fig. 2 $\bar{X}$ as a function of D for the GB+GB2 sample: *a*, for all sources; *b*, for the 'distant' ($z > 0.4$) sources.

Large Array (VLA) at 1,465 MHz with resolutions of 1–3 arc s. (Structural information on several other sources in the sample not mapped with the VLA is available in the literature.) Position angles are thus well determined for 48 and 97 sources in the GB and GB2 regions respectively. Pairs at different angular distances were formed independently in the two regions and the numbers in different bins of D were then combined to obtain the distribution of $\bar{X}$ shown in Fig. 2*a*. As optical identification data are again available for all the sources, the distant sample was formed, as for the Ooty sample, by omitting sources known to have $z < 0.4$ and those identified with galaxies of $m_{pg} < 20$. The distribution of $\bar{X}$ even for this restricted sample (35 sources in GB and 67 in GB2) does not depart significantly from 45° (Fig. 2*b*); for $D < 5^\circ$, we find $\bar{X} = 44.9^\circ$ for 194 pairs, but for smaller bin sizes, $\bar{X}(D < 2.5^\circ) = 38.8^\circ$ for 50 pairs and $\bar{X}(2.5^\circ < D < 5^\circ) = 47.1^\circ$ for 144 pairs, the difference in the first bin being significant only at the 1.7σ level. We note also that the low value of $\bar{X}$ for $D < 2.5^\circ$ is caused only by sources in the GB2 region which produce $\bar{X} = 33.7^\circ$ for 29 pairs (2.3σ), whereas for the GB sources alone $\bar{X} = 45.7^\circ$ for 21 pairs.

Having thus failed to confirm 'alignment' in our two samples, which are almost independent and in which the numbers of close pairs ($D < 5^\circ$) are comparable to those in the samples used by Sanders, we investigated a sample that should be similar to one of his and for which data are available in the literature. This is the heterogeneous sample obtained by combining well-mapped radio quasars with the 3CR sources. In forming the total sample we have, however, attempted to make it fairly homogeneous, taking care to avoid introducing any bias in the PAs of the radio axes. The PAs of the sources in the 3CR complete sample⁸ were determined either from the maps made with the Cambridge 5-km telescope (with an angular resolution of $2 \times 2 \cos \delta$ arc s at 5 GHz) or from maps of higher resolution available in the literature. Although several sources at low declinations have not been adequately resolved by this system, maps with higher angular resolution are available for most of these

(mainly from Cambridge observations at 15 GHz or from observations with the NRAO 3-element interferometer or VLA). As the upper limits to the values of largest angular size (LAS) for unresolved sources in the 3CR catalogue are now ≤ 4 arc s, we have considered only those having $LAS \geq 5$ arc s to avoid any discrimination against sources oriented largely in the north-south direction. Although all sources with redshift $z > 0.1$ were examined, those considered to be of FR class I (ref. 9) at $z < 0.1$ were excluded because their PAs are often ambiguous. The final 3CR sample contains 123 sources of which 76 have a measured or estimated $z > 0.4$.

Hintzen *et al.*¹⁰ have recently compiled a list of ~ 250 quasars for which structural information is available, including their own observations of ~ 120 quasars made using the VLA with a resolution of ≤ 3 arc s. It does not, however, form a complete sample in any well-defined sense and may not be entirely unbiased with respect to the orientation of the radio axes as it includes many sources at low declinations that have been mapped¹¹, using the Westerbork synthesis array, with poor resolution in declination. To make the sample as homogeneous as possible, we have considered only quasars included in the following four well-defined complete samples: (1) 4C quasars (observed by Potash and Wardle)¹²; (2) 4C quasars (Starnard and Neal)¹³ not included in the Potash-Wardle sample; (3) quasars from the Jodrell Bank 966-MHz survey (Owen *et al.*)¹⁴ and (4) 4C and Parkes samples (Wills)¹⁵.

All of these quasars (except a few in the Starnard-Neal sample) were originally observed with the NRAO 3-element interferometer at a resolution of ~ 3 arc s. We have therefore again considered only those having values of $LAS \geq 5$ arc s, which gives a total of 109 quasars (other than those in the 3CR) whose PAs could be estimated reliably from the best available maps or measurements.

The distribution of $\bar{X}$ for the total sample (3CR + quasars) shows no significant departure from a random distribution of PAs, with $\bar{X}(D < 5^\circ) = 44.3^\circ$. The results for the case of $z > 0.4$

Table 1 Values of $\bar{X}(D < 5^\circ)$ for different source samples and redshifts

Sample	$z > 0.1$				$z > 0.4$			
	No. of sources	No. of pairs	$\bar{X}$ (deg)	Significance*	No. of sources	No. of pairs	$\bar{X}$ (deg)	Significance*
(1) 3CR	108	36	37.8	1.67	76	23	39.1	1.09
(2) Sample (1) + quasars from refs 12–15	217	123	42.8	0.93	167	80	42.8	0.77
(3) Sample (2) + quasars from ref. 10	256	175	41.8	1.63	201	116	40.8	1.74
(4) Quasars alone from sample (2)	139	62	40.1	1.49	119	48	41.4	0.97
(5) Quasars alone from sample (3)	178	103	40.1	1.90	153	81	39.9	1.78

* In units of σ .

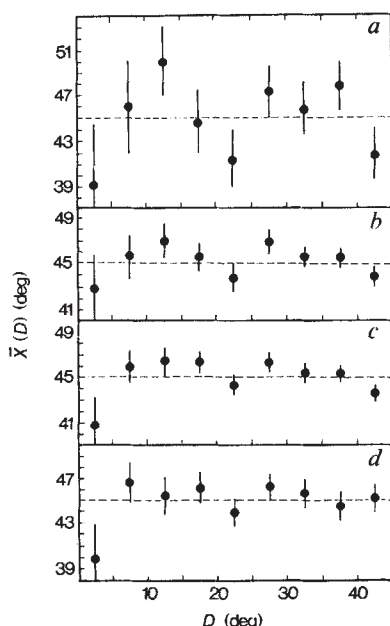


Fig. 3 Same as Figs 1 and 2, but for the 3CR+quasar samples with $z > 0.4$: a, 3CR alone; b, 3CR+quasars from complete samples (1)–(4) defined in the text; c, quasars from Hintzen *et al.* added to b; d, quasars alone from a, b and c.

are shown in Fig. 3, separately for the 3CR sample alone and after adding the quasars in the complete samples. The departures from $\bar{X} = 45^\circ$ for $D < 5^\circ$ are significant only at the $\approx 1\sigma$ level. Figure 3c also shows the distribution obtained by adding those quasars with $LAS \geq 5$ arc s and $z > 0.4$ mapped by Hintzen *et al.*¹⁰ with the VLA but not included in any of the complete samples (1)–(4). The addition of 34 such quasars increases the significance of the departure for $D < 5^\circ$ to $\sim 1.7\sigma$, which is clearly insufficient to establish the claimed 'alignment'. Note also that the quasars observed by Hintzen *et al.* do not form a homogeneous set.

The values of $\bar{X}(D < 5^\circ)$ for the different 3CR+quasar samples are summarized in Table 1 for two different lower bounds to the source redshifts. Notably, there is no systematic increase in the significance of the departures from $\bar{X} = 45^\circ$ as one increases the lower redshift limit from 0.1 to 0.4, as might be expected if the alignment was owing to gravitational imaging by superclusters, and increasing it beyond $z = 0.5$ reduces the significance even further.

The tendency towards axis alignment has also been found by Sanders² (for $D < 10^\circ$) in a sample of sources from the B3 survey, which was based on unpublished maps made using the VLA with an angular resolution of ~ 14 arc s. We note, however, that a proper analysis of this sample must await observations with higher angular resolution because many of the sources have total angular extents less than twice the half-power beamwidth. On the basis of our three almost independent large samples, we conclude that there is presently no significant evidence that the axes of distant radio sources tend to be aligned for angular separations $\geq 1^\circ$ on the sky.

Thermoluminescence and radiation damage in bismuth germanate

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Bismuth germanate ($\text{Bi}_4\text{Ge}_3\text{O}_{12}$ or 'BGO'), primarily because of its higher detection efficiency, is beginning to replace NaI(Tl) as a scintillator for detection of γ rays in several applications, including large electromagnetic calorimeters for high-energy physics experiments¹, computed tomography systems in medical physics² and γ -ray spectroscopy measurements in oil-well logging³. Although the use of BGO is increasing, the scintillation mechanism which converts incident γ -ray energy into visible light is not well understood and its efficiency (light output per unit energy of absorbed γ radiation) decreases following exposure to ultraviolet light or other radiation⁴. We have found that this ultraviolet 'radiation damage' is accompanied by a corresponding increase in a stored thermoluminescence signal—the first reported observation of thermoluminescence in BGO. Comparison of scintillation efficiency and thermoluminescence intensity following various exposures to ultraviolet light and after thermal annealing indicate a strong correlation between the scintillation efficiency and the electron population of the thermoluminescence traps. This should be a useful probe for investigating the scintillation mechanism of BGO.

Thermoluminescence is observed when electrons freed by the passage of ionizing radiation become trapped in metastable energy states between the valence and conduction bands of a crystal⁵. These traps are caused usually by impurity atoms or point defects in the crystal lattice and, if the crystal is heated sufficiently, the electrons will gain enough thermal energy to escape from the traps and may then recombine with holes at luminescence centres resulting in the emission of light. The temperature at which this occurs depends on the depth of the trap and consequently, as the sample is heated from room temperature to $\sim 400^\circ\text{C}$, electrons escaping from progressively deeper traps will produce signals at progressively higher sample temperatures. In contrast, scintillation is the prompt emission of visible light following some type of excitation⁶; when incident radiation is absorbed by the crystal, luminescence centres become excited and then return immediately to the ground state through the emission of photons.

The sample used for this study was a transparent colourless 6-mm diameter $\times$ 25-mm-long single crystal (Harshaw Chemical Co.). A 1-mm-thick slice was cut from one end for the thermoluminescence measurements and the remaining 6 mm $\times$ 24 mm crystal was used for the scintillation measurements. For the thermoluminescence measurements, the smaller sample was first irradiated with either ultraviolet or γ radiation and then heated from room temperature to 400°C in a dry nitrogen atmosphere at a rate of 8°C s^{-1} . The light emitted first passed through an infrared filter and then was detected with a bi-alkali photomultiplier and recorded as a function of sample temperature to produce the typical 'glow curve' form of thermoluminescence data. Scintillation measurements were made by coupling the larger crystal to a similar bi-alkali photomultiplier and observing the scintillation response to 662-keV γ rays from a 1- μCi ^{137}Cs source.

The basic thermoluminescence properties of BGO were investigated first. Figure 1 shows glow curves measured after exposing the sample to ultraviolet radiation from a mercury lamp, 60-keV γ rays from a ^{241}Am source and sunlight. The glow curves induced by ultraviolet exposure and γ irradiation are similar, whereas excitation by sunlight enhances the 150°C

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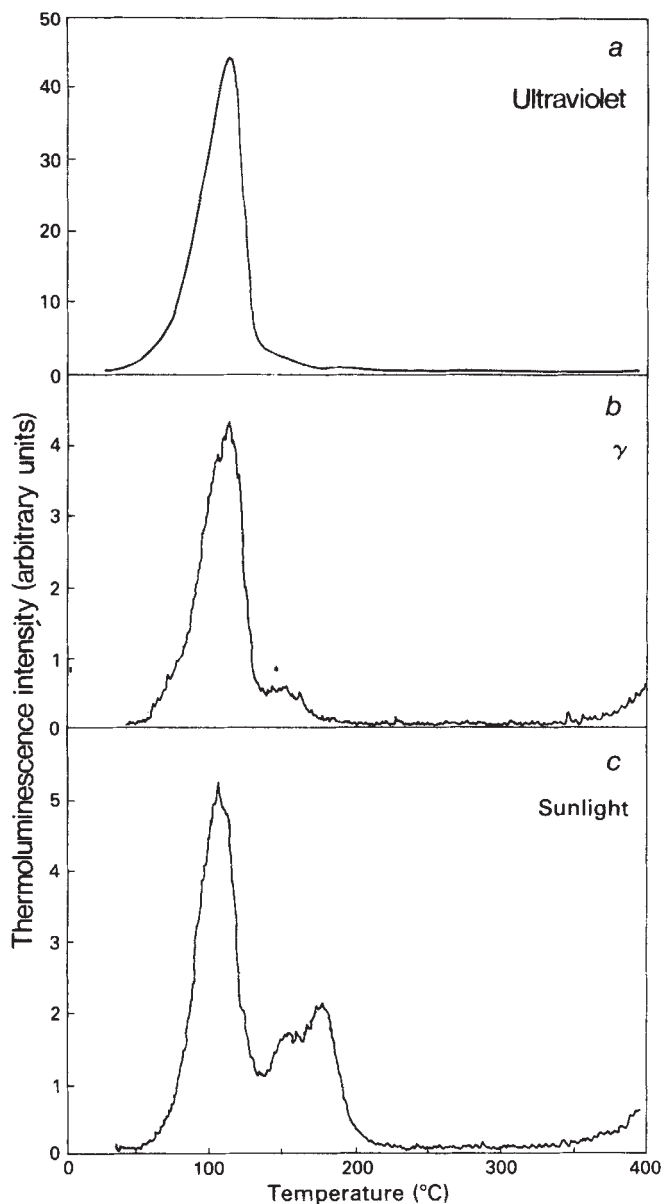


Fig. 1 Thermoluminescence glow curves of bismuth germanate following *a*, a 100-min ultraviolet irradiation at an irradiance of $40 \mu\text{W cm}^{-2}$, *b*, a 10-min exposure to 60-keV γ rays from a ^{241}Am source (the total dose was ~ 500 rad) and *c*, a 26-min exposure to sunlight. Four peaks representing four trapping levels can be identified. The dominant peak occurs at a glow curve temperature of 115°C with lesser peaks at 65, 150 and 175°C . The increase in light detected at temperatures above 350°C is caused by blackbody radiation, which is negligible at lower temperatures. The peak at 65°C is mostly obscured by the large 115°C peak and is seen only as a shoulder on the low temperature side of the 115°C peak. The 65°C peak is seen only when the sample is measured immediately after irradiation as the population of this trap decays rapidly at room temperature.

and 175°C peaks relative to the 115°C peak, the reason for which, although not understood, may be related to the excitation wavelength. The sensitivity (thermoluminescence intensity/radiation dose) of the 115°C peak to γ irradiation is $\sim 0.1\%$ of that of the 195°C peak in LiF.

The trapping level responsible for the 115°C peak was measured by the isothermal decay method⁷, in which the sample is irradiated (in this case with ultraviolet) and then stored at a constant temperature T_1 . The decay of the thermoluminescence is then measured as a function of time and the process then repeated for a second storage temperature T_2 . If the decay is exponential (that is, if the thermoluminescence kinetics are first

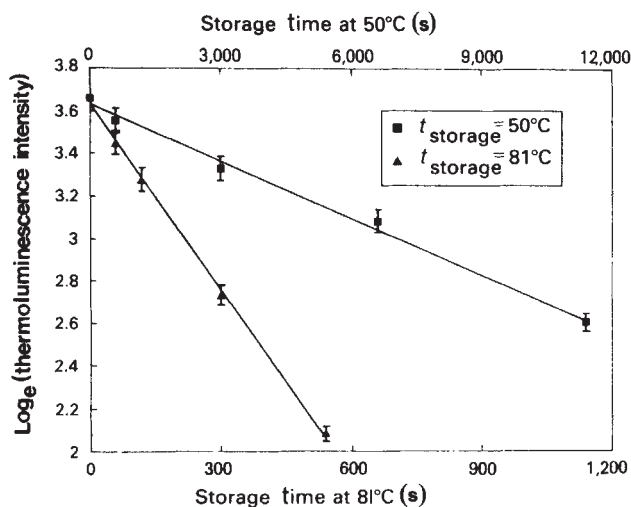


Fig. 2 Decay of the intensity of the 115°C thermoluminescence peak in bismuth germanate following a 30-min ultraviolet irradiation. The decay time τ at a storage temperature of 50°C was 11,300 s; the decay time at 81°C was 340 s.

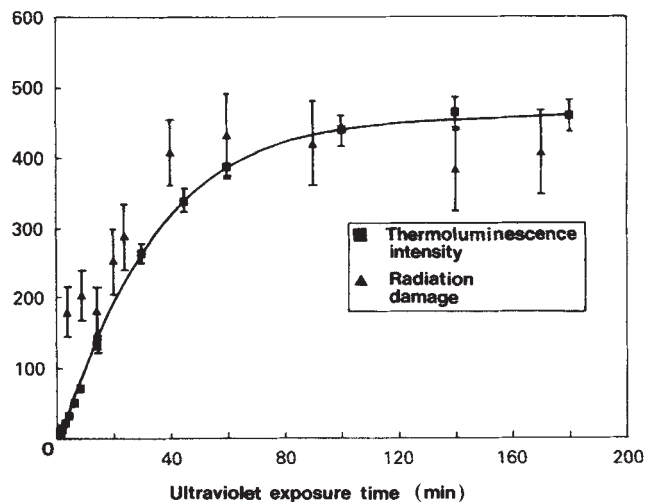


Fig. 3 Thermoluminescence intensity of the 115°C peak and radiation damage (reduction in scintillation output) as a function of ultraviolet exposure time. The units on the y-axis are arbitrary. (The irradiance of the Hg lamp is $40 \mu\text{W cm}^{-2}$ at the sample position; 90% of the flux is emitted at 253.7 nm .) A saturation level is reached after about 1 h beyond which additional irradiation results in little increase in the thermoluminescence intensity or radiation damage. (The 150 and 175°C peaks seem to saturate sooner.)

order) then the trap depth, E , can be calculated from

$$E = \frac{k}{1/T_1 - 1/T_2} \ln\left(\frac{\tau_1}{\tau_2}\right) \quad (1)$$

where τ_1 and τ_2 are the decay times to $1/e$ of the initial intensity at storage temperatures T_1 and T_2 and k is the Boltzmann constant. Figure 2 shows the results of isothermal decay measurements for storage temperatures of 50°C (323 K) and 81°C (354 K). The decay is exponential, indicating that the technique is applicable. A trap depth of $1.1 \pm 0.1 \text{ eV}$ is calculated from decay times of 340 s at 81°C and 11,300 s at 50°C . The effect of overlapping peaks on this determination is negligible owing to the large relative intensity of the 115°C peak. The frequency factor, s , calculated from $\tau = s^{-1} \exp(E/kT)$ is $1.2 \pm 0.1 \times 10^{13} \text{ s}^{-1}$.

The scintillation efficiency of BGO decreases by up to 50% following exposure to ultraviolet light or γ radiation or sunlight, which could result from electrons, freed by the radiation, becoming trapped at metastable energy levels. If these electrons are

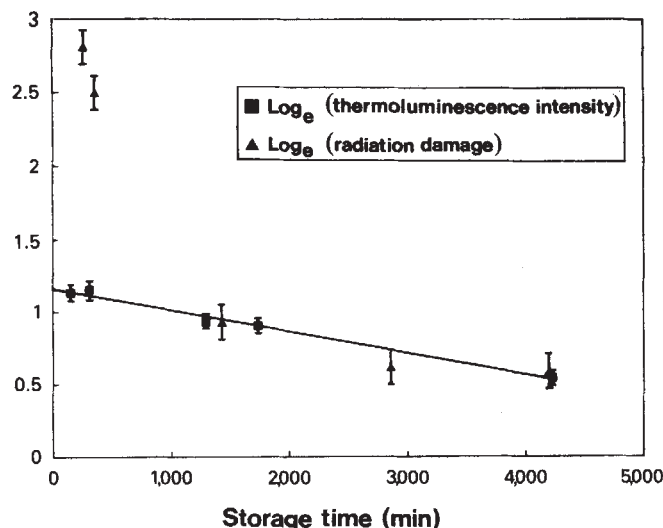


Fig. 4 Decrease in the thermoluminescence intensity of the 115 °C peak and decrease of radiation damage (reduction in scintillation output) for samples stored at 22 °C following a 30-min ultraviolet exposure.

normally involved in the scintillation process, then the scintillation output will be reduced in proportion to the fraction of these electrons that become trapped and the filling of these metastable energy levels will be similar to the mechanism which stores thermoluminescence. Thus, we looked for correlations between the thermoluminescence traps and the traps responsible for radiation damage, first by comparing the behaviour of thermoluminescence and radiation damage as a function of radiation exposure. Figure 3 shows the intensity of the 115 °C peak as a function of ultraviolet irradiation time and also the radiation damage induced in the 6 mm × 24 mm sample by the same exposure, defined as the reduction in the scintillation response to 662-keV γ rays following the ultraviolet exposure. Although the error bars are large, the radiation damage appears to follow about the same pattern as the thermoluminescence, saturating after about the same exposure, except that the radiation damage is larger than the thermoluminescence for short exposures, suggesting a contribution from an additional trapping centre.

We compare also the decay of the thermoluminescence to annealing of radiation damage in BGO. Scintillation efficiency, reduced by ultraviolet, γ or sunlight exposure, will recover subsequently if the crystal is stored at room temperature for several days, or at an elevated temperature for a shorter time, which is similar to the decay of thermoluminescence. Thus a second technique was used to investigate the possible connection between thermoluminescence and radiation damage. Both the thermoluminescence sample and the scintillator sample were irradiated with ultraviolet for 30 min, which resulted in a 11% decrease in the scintillation efficiency (light output) and also stored a thermoluminescence signal. The samples were stored at room temperature and the scintillation efficiency and thermoluminescence intensity of the 115 °C peak were measured as a function of time. Figure 4 shows the decay of the thermoluminescence signal and the simultaneous recovery of the scintillation efficiency (plotted as the decrease in radiation damage), which, for times greater than 1,000 min, occur at the same rate, again suggesting a correlation between these phenomena. But for shorter times the radiation damage decreases more rapidly, probably owing to the depopulation of a shallower trap, perhaps the one observed at 65 °C; preliminary measurements of the radiation-damage decay time of a sample stored at 10 °C, together with the data in Fig. 4, suggest a depth of approximately 0.9 eV for the shallow trap, which is approximately the depth expected for the 65 °C peak, although it was not possible to determine it directly by thermoluminescence measurements. Thus, the population of this shallow trap is

probably related to the short term recovery of radiation damage, whereas the population of the 1.1 eV trap (115 °C peak) is related to the longer term recovery of radiation damage.

Two conclusions may be drawn from these initial results. First, BGO displays a relatively-strong thermoluminescence signal with reasonably well-defined peaks. The 115 °C peak readily lends itself to a detailed investigation of decay time, trap depth and frequency factor, but the other peaks will require thermal or optical bleaching or perhaps slower heating rates to isolate them for further study. Second, the measurements reported here suggest that the thermoluminescence traps and the radiation damage traps may be related. In particular, both thermoluminescence and radiation damage (that is, reduced scintillation efficiency) are produced by the same type and intensity of excitation (ultraviolet, γ and sunlight), both quantities increase with increasing ultraviolet exposure and saturate after similar exposures and both quantities decay at the same rate following excitation, which suggests that the thermoluminescence traps and the radiation damage traps are indeed related and may in fact be the same traps. If this is indeed true, then thermoluminescence could prove to be a valuable tool for studying both the radiation damage mechanism and the basic scintillation mechanism itself, the main advantage being its high sensitivity. Further investigation of the correlation between the 65 °C peak and short-term radiation-damage recovery and comparison of thermoluminescence properties with trace element compositions of different BGO crystals should provide further insight into the relationship between these effects in BGO.

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Dirty snow after nuclear war

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The notion that smoke from fires started by nuclear explosions could alter the Earth's climate¹ is supported by quantitative models of climate^{2-5,27} showing that severe cooling may be expected at continental surfaces in the first few months following a full-scale nuclear war, because of the reduced transmission of sunlight through the atmospheric smoke. Whether or not these model results are correct, we show here that the smoke could continue to cause significant climatic disruption even after it has fallen from the atmosphere, by lowering the reflectivity of snow and sea-ice surfaces, with possible effects on climate in northern latitudes caused by enhanced absorption of sunlight. Indeed, on Arctic sea ice and on the ablation area of the Greenland ice sheet, the reduced reflectivity could persist for several years.

The fraction of light reflected by pure snow is >90% at visible wavelengths, but much lower in the near-infrared. The spectral measurements can essentially be explained by radiative-transfer modelling⁶. As convective and latent-heat transfers over snow-packs are, typically, smaller than the radiative fluxes⁷, the

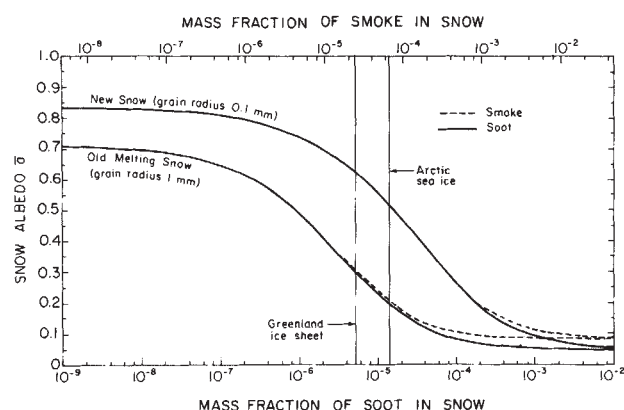


Fig. 1 Spectrally-averaged snow albedo as a function of soot (bottom scale) or smoke (top scale) content, for two different snow grain sizes. (The grain size is the radius of an optically-'equivalent' sphere and is related to the volume/surface ratio of nonspherical snow grains¹¹.) The spectral complex refractive index of ice is obtained from ref. 24. 'Soot' is assumed to have complex refractive index $1.8 - 0.5i$, independent of wavelength, and density 1.0 g cm^{-3} (footnote 3 of ref. 9) and to be present as uniform spheres of radius $0.1 \mu\text{m}$. These values imply a mass absorption coefficient for soot decreasing with wavelength from $11.7 \text{ m}^2 \text{ g}^{-1}$ at $\lambda = 300 \text{ nm}$ to $4.6 \text{ m}^2 \text{ g}^{-1}$ at $\lambda = 1,000 \text{ nm}$. 'Smoke' also has density 1.0 g cm^{-3} ; its refractive index, $1.55 - 0.1i$, was specified by NAS¹⁴ for visible wavelengths, but is also used here for the near-infrared. This assumption is not crucial, because the major reduction of albedo is at visible wavelengths; pure snow already has low albedo in the near-infrared because of the greater absorptivity of ice there. The smoke has log-normal size distribution with number-mode radius $r_m = 0.1 \mu\text{m}$ and log-normal width $\gamma = 2.0$ (Table 5.7 of ref. 14). This distribution has effective radius²⁵ $r_{\text{eff}} = 0.33 \mu\text{m}$. Doubling the mode radius to $r_m = 0.2 \mu\text{m}$ ($r_{\text{eff}} = 0.66 \mu\text{m}$), to simulate possible aggregation of the smoke particles, would have the same effect as reducing the concentration of smoke in snow by a factor of 1.5. Calculation is for an 'external mixture' of soot particles and ice particles; for an internal mixture the curves should be shifted by a factor of ~ 2 to the left. The incident solar radiation spectrum is assumed to be that for subarctic summer, clear sky, solar zenith angle 53° , at sea level. A given concentration of impurities would reduce albedo somewhat more under a cloudy sky. Vertical lines give concentrations for the scenarios discussed in the text, for smoke distributed through one month's snowfall.

energy budget of the snowpack is strongly influenced by the spectrally-averaged albedo

$$\bar{a} = \frac{\int_0^\infty a(\lambda) S(\lambda) d\lambda}{\int_0^\infty S(\lambda) d\lambda} \quad (1)$$

where $a(\lambda)$ is the snow albedo (ratio of upward to downward radiation flux) at wavelength λ and $S(\lambda)$ is the spectral distribution of solar energy flux incident on the surface ($\text{W m}^{-2} \mu\text{m}^{-1}$). Pure snow reflects 70–85% of the solar energy incident on it, depending primarily on the snow grain size which normally grows as the snow ages⁸; $\bar{a}$ is 80–85% for new snow of grain radius r in the range 50–100 μm and $\bar{a} \approx 70\%$ for old melting snow ($r \approx 1 \text{ mm}$). The albedo is also influenced by solar zenith angle and by cloud cover.

Small amounts of absorptive impurities in snow can reduce the albedo dramatically in spectral regions where it is high (visible wavelengths). We previously computed⁹ the radiative effects of graphitic carbon, 'soot' (mass fraction $\leq 10^{-7}$) distributed uniformly through a snowpack, and subsequent experiments, measuring both albedo and soot content¹⁰, agreed within the experimental uncertainty (a factor of ~ 2 in soot content) with predictions of our radiation model¹¹.

For the present purpose, the earlier results (Fig. 7 and footnote 3 of ref. 9) must be extended to larger amounts of soot and also be averaged over wave-length. The solar spectrum at the Earth's

surface, $S(\lambda)$, is obtained using Wiscombe's atmospheric radiation model¹² for the subarctic-summer standard atmosphere¹³. Figure 1 shows the dependence of snow albedo on soot content, for two values of snow grain size. A given amount of soot causes a greater reduction in albedo in old snow than in new snow because the radiation penetrates deeper on average in old coarse-grained snow and therefore encounters more absorbing material before being scattered back out of the snowpack.

Results are also shown of calculations using the optical properties of the 'smoke' for the baseline nuclear war scenario of the US National Academy of Sciences (NAS)¹⁴. As this smoke contains 20% soot and 80% transparent oily compounds, it is less absorptive than pure soot, but the effects of a given amount of 'smoke' on snow albedo closely match those calculated for pure soot of the amount present in the smoke. Thus, in Fig. 1, by shifting the top scale (for smoke) by a factor of ~ 5 relative to the bottom scale (for soot), we were able to superimpose the results for 'smoke' on those for 'soot', except in very polluted snow (dashed curves).

All these calculations are done by modelling soot or smoke in snow as an 'external mixture' (impurity particles separated from ice particles), which may underestimate the true effect of the impurities as a given reduction of albedo can be achieved by about half as much soot, if the soot is instead located inside the ice grains^{15,16} ('internal mixture'). (However, the snow albedos, $\bar{a}$, of Chylek *et al.*¹⁵, are too low because, for $S(\lambda)$ in equation (1), these workers used the solar spectrum at the top of the atmosphere instead of at the snow surface; for example, the absorptivity of pure snow with $r = 0.1 \text{ mm}$ should be 0.17 (Fig. 1 here) instead of 0.21 in their Fig. 7.) The most probable situation, in which smoke is scavenged by falling snow crystals, ending up on the surface of the ice grains¹⁷, would probably give results intermediate between those of external and internal mixtures.

We now use Fig. 1 to estimate the reduction of snow albedo following nuclear war for the case of snow covering the sea ice of the Arctic Ocean, the surrounding tundra of the northern continents and the Greenland ice sheet, areas where the absorption of sunlight would be most enhanced because the effect of changing snow albedo is not muted by forest cover. We use the NAS¹⁴ baseline scenario in which $1.8 \times 10^{14} \text{ g}$ of smoke is injected into the atmosphere. Because of its proximity to sources of smoke, the Arctic may suffer more pollution than the average for the Northern Hemisphere, but here we simply assume the smoke is uniformly deposited over the Northern Hemisphere, so the total smoke fallout is 0.71 g m^{-2} . The e-folding decay time for smoke fallout is probably in the range 10–30 days. (For the baseline scenario in Fig. 1 of ref. 2 it is 30 days.) Using an average precipitation of $13 \text{ g cm}^{-2} \text{ yr}^{-1}$ for the Arctic Ocean¹⁸ and $37 \text{ g cm}^{-2} \text{ yr}^{-1}$ for Greenland¹⁹, the mass fraction of smoke in the snow would be 6.5×10^{-5} on the Arctic sea ice and 2.2×10^{-5} on Greenland, if distributed through one month's precipitation in each case. These concentrations are indicated by vertical lines in Fig. 1. The values of $\bar{a}$ intersected by these lines indicate that the fraction of solar energy absorbed by snow ($1 - \bar{a}$) on the Arctic Ocean would increase by a factor of 2.8 (new snow)–2.7 (old melting snow) and on Greenland by a factor of 2.2–2.4, respectively. But a net positive effect on the snow energy budget cannot be expected until most of the soot has fallen from the atmosphere so that it no longer blocks the sunlight.

The estimates of smoke concentration in the snow depend, of course, on the snowfall rate and the smoke sedimentation rate and on the total smoke injection into the atmosphere, all of which are highly uncertain. The snowfall rate may be reduced, for example, because of the greater atmospheric stability in the presence of smoke². The vertical lines in Fig. 1, therefore, are only illustrative of the possible magnitude of the effect and are not meant to be quantitative predictions. Other scenarios for different amounts of smoke can be read off Fig. 1, as desired. For example, if the total amount of smoke were the same as we assumed, but fell over a period of two months instead of one

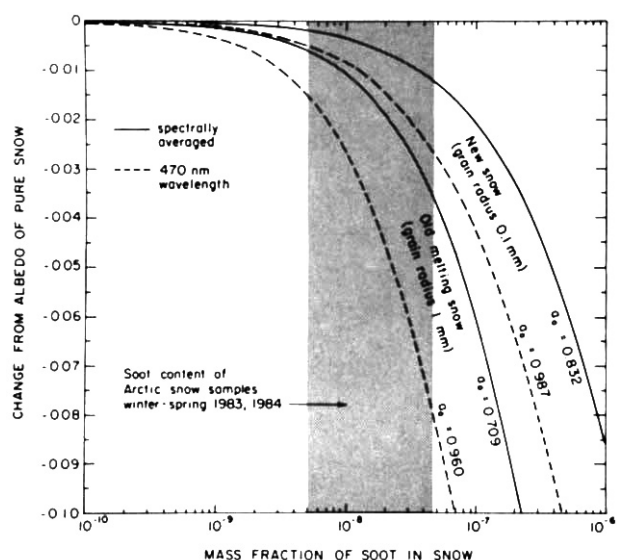


Fig. 2 Computed effects on snow albedo caused by small mass fractions of soot. Soot size distribution and refractive index, snow grain sizes and solar radiation spectrum are the same as used in Fig. 1. The changes from the albedo values of pure snow, a_0 , are plotted; the spectrally-averaged changes (solid lines) correspond to the left-most portion of the plots in Fig. 1, on an expanded vertical scale here. The dashed lines are calculations at the wavelength where snow albedo is most sensitive to soot content ($\lambda = 470$ nm). The reduction in spectrally-averaged albedo is thus approximately half that at visible wavelengths. The shaded region indicates the range of soot concentrations determined²⁶ in 12 samples of snowfall collected from Arctic Canada, Alaska, Greenland and Svalbard during winter and spring 1983-84. To ensure consistency between soot measurement and albedo calculation, they have been multiplied here by the factor 0.85; previously²⁶, a mass absorption coefficient $k_{\text{abs}} = 8.5 \text{ m}^2 \text{ g}^{-1}$ for ambient soot at $\lambda = 525$ nm was assumed, whereas the Mie calculation for the soot parameters used here gave $k_{\text{abs}} = 10.0 \text{ m}^2 \text{ g}^{-1}$.

(or if it fell quickly, but was distributed through two months snowfall by wind-drifting), the vertical lines should be moved a factor of two to the left, changing the albedo of polluted new snow on Arctic sea ice from 0.52 to 0.60; of old snow from 0.20 to 0.27.

These hypothetical effects of nuclear war are all much larger than the possible reduction of snow albedo of a few per cent caused by the small concentrations of soot found at present in Arctic snow (Fig. 2).

In the above four cases (intersections of lines in Fig. 1), the polluted layer has sufficient optical thickness for the albedo to be unaffected by the presence of clean snow beneath. However, light penetrates clean snow more deeply than polluted snow. Figure 3 shows how the albedo recovers to high values as the smoke layer is covered by clean snow, assuming for the smoke layer the lowest albedo of the four cases, $\bar{a} = 0.203$. The thickness of clean snow necessary to hide the smoke layer, such that the smoke retains only a 1% effect on $\bar{a}$, is 2 g cm^{-2} for new snow and 10 g cm^{-2} for old melting snow (corresponding to about 2 and 10 months precipitation in the Arctic, respectively); this means, of course, that as the clean snow ages, its hiding power diminishes.

Although the albedo would rise as clean snowfalls bury the smoke, the polluted layer may be exposed again during a subsequent melt-season and so hasten the disappearance of the snow from the Arctic tundra and sea ice. Experiments are needed to determine whether small smoke particles tend to concentrate at the surface of melting snow, as do micrometre-size dust particles²⁰.

At high altitude locations on the Greenland ice sheet, the incident solar spectrum $S(\lambda)$ is somewhat different from that used here (compare Fig. 1 caption) but, judging from high-altitude Antarctic calculations²¹, the difference in $\bar{a}$ is small

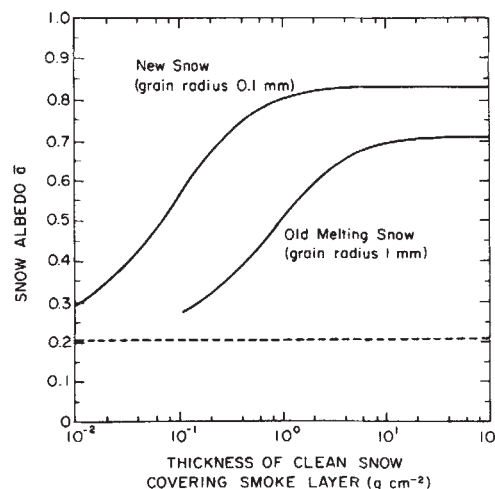


Fig. 3 Spectrally-averaged snow albedo as a function of the thickness of clean snow covering the smoke layer, for two different snow grain sizes. The smoke is assumed to be distributed through a layer of coarse-grained snow, $r = 1$ mm, corresponding to one month's snowfall in the Arctic Ocean, so that it has spectrally-averaged albedo 0.203 when on the surface (intersection of vertical line 'Arctic sea ice' with dashed curve in Fig. 1, shown here as horizontal dashed line). Solar radiation spectrum is the same as for Fig. 1. No calculations are done for snowpack thicknesses less than a monolayer of snow grains.

enough for these figures still to be used. Unlike the Antarctic ice sheet, where temperatures remain far below freezing throughout the year, in Greenland there is normally some melting in summer over 70% of the ice sheet^{19,22}. This melting could be enhanced by smoke fallout, especially in the ablation area of the Greenland ice sheet (~15% of the ice sheet area) where the smoke would be exposed repeatedly in subsequent summers. The effect on the mass budget of Greenland may be estimated from Ambach's model²³.

Persistent consequences would also be expected on multi-year Arctic sea ice. Because the snow now melts completely every summer on Arctic sea ice and because of the direction of sea-ice growth (by freezing at the bottom), the smoke layer would probably be uncovered repeatedly in subsequent summers to darken the ice.

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Correlative 90 kyr northeast Asia–northwest Pacific climate records

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Specific regional and hemispheric responses to past variations in solar radiation demonstrate the complex interconnections that exist between components (land/sea/atmosphere) of the climate system^{1,2}. Few empirical palaeoclimatic studies are available from northeastern Asia, a region essential for the construction of composite models of global climate change and critical to analyses of northwestern North American climate. Among these, Quaternary climatic data from Japan are restricted largely to pollen sequences <30 kyr BP, as limited chronostratigraphic control constrains older data³⁻⁶. Palaeoenvironmental studies of the northwest Pacific Ocean focus on comparing modern conditions with the last glacial maximum⁷. Continuous marine palaeoclimatic series are far from land and are not correlated directly with continental sequences^{8,9}. Here we present the first continuous records of vegetation and climate from northeastern Japan over the past 90 kyr, relating them directly to sea-surface temperature (SST) estimates from the same deep-sea core, RC14-103 located in the northwest Pacific Ocean east of Hokkaido (Fig. 1). Relative stable glacial (~80–20 kyr BP) environments are cold (<4 °C) and wet (~1,000 mm), with boreal forest and tundra on Hokkaido associated with cool (<16 °C) summer and cold (<1.0 °C) winter SSTs offshore; non-glacial (~10–4 kyr BP) environments on land are warm (>8 °C) and humid (>1,200 mm), whereas SSTs are cold (10.4–14.3 °C) in summer and warm (>1.5 °C) in winter.

Modern pollen and radiolaria distributions in sediments from seas around Japan are used to interpret fossil floral and faunal changes^{10,11}. Specifically, factor analysis of abundance data for 35 radiolarian species in 66 surface sediment samples from the northwest Pacific⁸ yielded four assemblages accounting for 95% of the distribution variance in the data set. The four radiolarian factors correlate with surface water masses for which they are named: subtropical (Subtropic Gyre), subpolar (Subpolar Gyre), transitional (Subarctic Front) and Okhotsk (Sea of Okhotsk). Transfer functions developed from regression analyses relating these radiolarian assemblages to observed SSTs have standard errors in the estimates for winter and summer of 1.3 °C and 0.9 °C respectively. The SSTs derived from these radiolarian assemblages and vegetation/climate changes inferred from pollen assemblages in the core are correlated directly with regional and global stratigraphies¹²⁻¹⁴. Time control is based on radiolarian stratigraphy developed for the northwest Pacific¹⁰. In RC14-103, two distinct modes separated by a brief transition appear in floral and faunal down-core assemblages: (1) a post-glacial mode (11 kyr BP–present), distinguished by oak-dominated broadleaf pollen and subpolar radiolarian assemblages and (2) a glacial mode (~80–18 kyr BP) characterized by spruce-dominated conifer pollen and 'Sea of Okhotsk' radiolarian assemblages (Fig. 2).

Maximum concentrations of pollen from warm temperate oak forests occurred between ~11 and 4 kyr BP, whereas spruce,

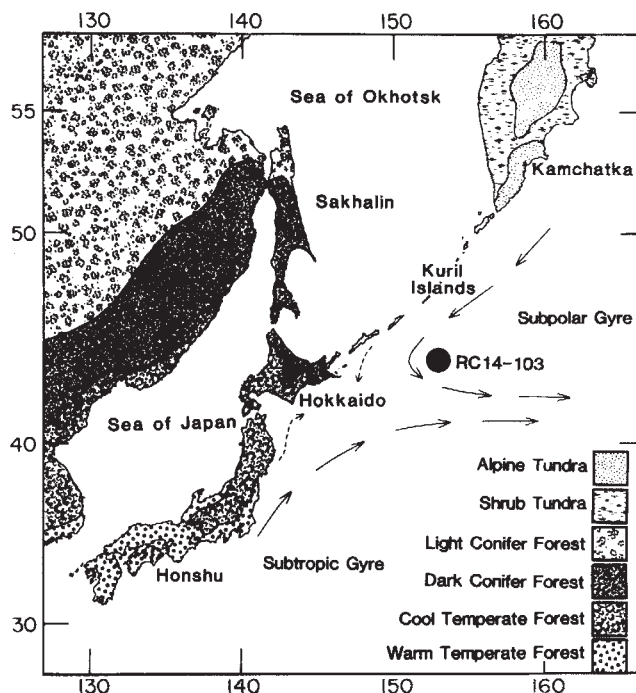


Fig. 1 Location of core RC14-103 (44°02' N, 152°56' E, 5,365-m water depth) in the northwest Pacific–northeast Asian study area. The core site, in the southwest portion of the Subpolar Gyre, is just north of today's Subarctic Front (confluence of currents from Subtropic and Subpolar Gyres). The flow of major surface currents in the region is indicated by arrows. Schematic vegetation distribution is modified from Tsukada²⁸, Walter²⁸ and Wang²⁹. Major Japanese vegetation groups are associated closely with latitudinal and altitudinal temperature variation. Mean annual temperatures of evergreen broad-leaved (warm temperate), deciduous broad-leaved (cool temperate) and boreal/subalpine (dark) conifer forests are 13–21 °C, 6–13 °C, and <6 °C respectively³⁰.

hemlock (*Tsuga*), birch (*Betula*) and alder (*Alnus*) were insignificant. Climatic conditions of the youngest 4 kyr approached those of the present, with increased spruce, Japanese cedar (*Cryptomeria japonica*) and *Sphagnum* reflecting the cool moist environments of northeastern Japan. Between 18 and 11 kyr BP, birch-alder and conifer (subarctic and cold temperate) factors were prominent, with the abundance of pine and the occasional absence of Japanese cedar suggesting climates which were periodically cool and often dry^{15,16}. Full glacial (25–18 kyr BP) sediments contain maximum amounts of boreal (spruce-dominated), park-tundra and tundra (including, sedge (Cyperaceae) and *Sphagnum*). High concentrations of *Selaginella selaginoides*, a common arctic and alpine tundra species^{17,18}, indicate that cold subarctic conditions prevailed on northern Hokkaido during this time interval. From ~60 to 25 kyr BP, slight expansion of oak and birch-alder assemblages, together with the intermittent presence of Japanese cedar, suggests there were minor variations in precipitation and temperature within an overall cold dry climate. Boreal conifers (primarily spruce), low frequency of broad-leaf taxa and a significant increase in Japanese cedar and hemlock (trees associated with cool moist environments) characterize the interval ~80–60 kyr BP. In the oldest sequence in RC14-103 (~90–80 kyr BP), pollen consists mainly of pine, oak, birch and alder, with few Japanese cedar and boreal conifers, indicating slightly warmer drier summers than in the glacial period.

Estimated SSTs vary from 3.2 °C below to 1.4 °C above present winter and 6.2 °C below and 3.0 °C above present summer temperatures (Fig. 2). The warmest Holocene SSTs are recorded for ~6–4 kyr BP. Early Holocene winter SSTs, ~1.5 °C below the present temperature, rise during the postglacial climatic optimum to values similar to the February temperature of today. Low early-Holocene summer SSTs reach a maximum at 5 kyr BP.

Late-Holocene winter and summer SSTs decrease from their climatic optimum highs, becoming warmer again during the past 1,500 yr. During the Holocene–Pleistocene transition, summer SSTs estimates are close to present summer temperatures, with winter SSTs more characteristic of cold full-glacial values. Between 60 and 18 kyr BP glacial winter SSTs consistently average 2 °C cooler than present, whereas summer SSTs vary within a 4 °C range, with an upper limit comparable to the present summer temperature at this site. From 80 to 60 kyr BP, winter SSTs are slightly cooler (~1 °C) and summer SSTs are close to the observed seasonal values. Summer SSTs average 1.5 °C warmer and winter SSTs are generally slightly cooler for 90–80 kyr BP.

Climatic changes in the northern Japanese Archipelago are broadly similar to contemporaneous climatic changes elsewhere in northeastern Asia^{3–6,17–21}. In northern Hokkaido, as in central Japan, full development of thermophilous vegetation is restricted to the Holocene, and estimated average annual temperatures increase ~2 °C during the climatic optimum^{17–20}. Pollen data in core RC14-103 also indicate there was less summer precipitation in Hokkaido at the climatic optimum than in preceding and subsequent millennia. Cold climates generally prevailed in northern Japan between 80 and 18 kyr BP. Although boreal conifers dominated the glacial period, occasional minor increases in summer temperature can be inferred from fluctuations of temperate pollen taxa. In northeastern Japan, interstadial temperatures and precipitation appear to have been comparable with modern conditions on the southern Kuril Islands, whereas stadial temperatures and precipitation were comparable with conditions on southern Sakhalin^{22,23}.

Early Holocene (10–7 kyr BP) low summer SST estimates suggest a weakened influence of the Kuroshio Current (western and northern extensions of the Subtropic Gyre) at the core site, with relatively little seasonal latitudinal variation in the Subarctic Front centred at 41° N. Full glacial SSTs persisted from 60 to 18 kyr BP. Large seasonal variations in the Subarctic Front and in SST (a difference of 16 °C between February and August) occurred during the glacial period owing to southward penetration in winter of an intensified Oyashio current (western and southern extensions of the Subpolar Gyre).

During the last glaciation, radiolarian assemblages and SSTs in the Pacific Ocean at the RC14-103 site were similar to those characteristic of the Sea of Okhotsk today, whereas vegetation and climates of northeastern Hokkaido appear comparable with those of modern southern Sakhalin and the southern Kuril Islands (Fig. 1). Through most of the last glacial, land and marine environments of northeastern Asia showed comparable south-southeastward displacement. This positive correlation between ocean and land temperatures was reversed during much of the Holocene, when warm summer temperatures in Hokkaido were contrasted with cold summer SSTs offshore. Marine and land data agree in that conditions in both environments least resemble the present conditions in and around the Sea of Okhotsk. Intensified Asian monsoon circulation may account for the estimated increase in land temperatures accompanied by lower SSTs²⁴.

An important aspect of marine and terrestrial climatic data from RC14-103 is the difference between these curves (Fig. 2) and other 90-kyr proxy climate records, most of which resemble the oxygen isotope curve, with some degree of offset^{1,25–27}. In RC14-103, summer SSTs do not conform to global climate patterns. Although winter SSTs and pollen data show mid-Holocene temperature maxima, and near-freezing SSTs and subarctic conditions prevailed in Hokkaido during the last glaciation, the curves reflect little of the climatic changes described in records of lower resolution. Whereas differences between the various curves of pollen assemblages may be an artefact of data resolution, they undoubtedly reflect differences in proximity to ice sheets of North America and Europe. The Japanese Island Arc, surrounded by the Pacific Ocean and the Sea of Japan, would be less sensitive to change in continental ice sheets and more sensitive to local marine and atmospheric changes.

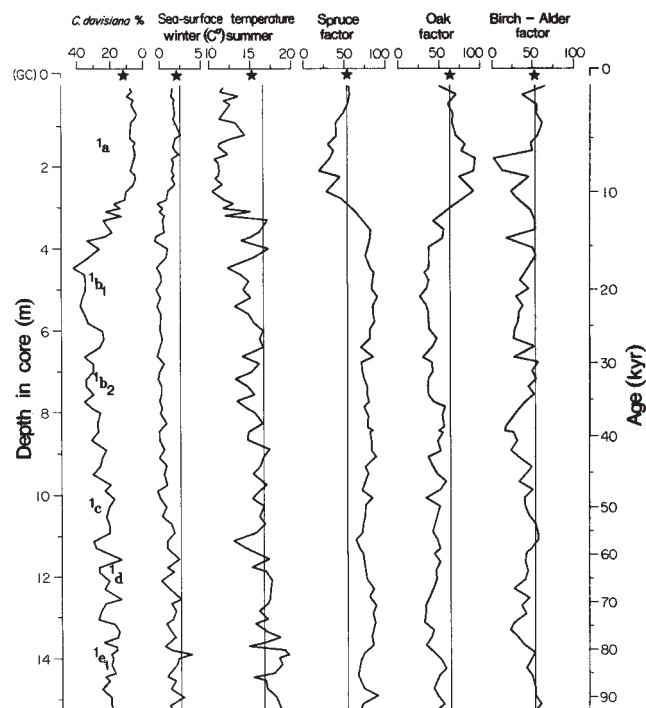


Fig. 2 Depth plots of parameters measured in piston and gravity core (GC) RC14-103: relative frequency of *Cycladothoira davisiana* (% total radiolaria) with designated maxima (¹b₁) and minima (¹a) levels, winter and summer SSTs, relative importance of spruce, oak and birch-alder factors (factors loadings ×100), and chronology. Stars designate present-day values in gravity core (GC) sample 0–5 cm. Analyses of additional GC samples indicate that sediment in the piston core top is not representative of the most recent conditions. Vertical reference lines for winter and summer SSTs represent the modern SSTs for February (2.5 °C) and August (16.6 °C) at the core site; those of the three pollen assemblages represent modern (GC) values. Chronology for RC14-103 is derived from continuous *C. davisiana* stratigraphy and the *Lynchoanum grande* extinction, which are correlated with the global oxygen isotope stratigraphy^{12–14}. Core samples taken at ~1,200-yr intervals were processed using standard techniques. Faunal and floral data (>250 radiolaria and 300 pollen) were synthesized by Q-mode factor analysis producing factors accounting for 95% of each data set. Pollen factors correspond with major vegetation formations of northeast Asia^{10,11}. Estimates of winter and summer SSTs vary between –0.7 °C and 3.9 °C for February and 10.4 °C and 19.6 °C for August.

We conclude that marine and terrestrial records from RC14-103 reflect dynamic interaction between northeastern Asian atmospheric and oceanic systems overprinting global climatic signals. These detailed climatic reconstructions of the past 90 kyr in the northwest Pacific and northeastern Japan provide the first comprehensive framework for examining effects of variations in atmospheric circulation, solar insolation and other components of the climate system in north-east Asia.

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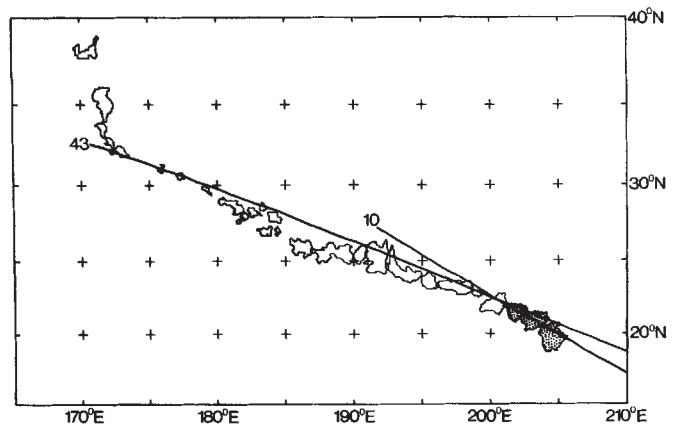


Fig. 1 Hawaiian seamount chain delineated by 2,000-fathom contours. 43, Trend of chain predicted by the Euler pole² found using Pacific plate hotspots with ages of 43-0 Myr BP. 10, Trend of chain predicted by the Euler pole³ found using Pacific plate hotspots with ages of 10-0 Myr BP. Stippled areas, volcanic edifices <5 Myr old.

Change in motion of Pacific plate at 5 Myr BP

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Alignments of volcanoes such as the Hawaiian seamount chain record the passage of oceanic plates over mantle plumes or hotspots, and changes in the orientation of a seamount chain may indicate changes in the direction of motion of the plate within the hotspot frame of reference¹. This hypothesis can be tested for the Pacific plate by comparing data from the Hawaiian volcanoes with tectonic movements inferred from the geology of adjacent plates. We have now found that the motion of the Pacific plate changed to a more northerly direction 5 Myr ago, probably as a result of the detachment of a piece of Pacific sea floor which had been subducted beneath the Fiji plateau during the Tertiary.

The change of motion is depicted in Fig. 1. The curves labelled '43' shows the trend of the Hawaiian seamount chain predicted by an Euler pole² for Pacific plate-hotspot (PA-HS) motion determined from many Pacific hotspot tracks spanning the past 43 Myr. The curve labelled '10' shows the trend predicted for the eastern end of the chain by an Euler pole found from PA-HS tracks spanning 10 Myr or less³. A shift in PA-HS motion at any time within the interval 10-0 Myr BP could produce a shift like that seen between the 43-0 Myr BP and 10-0 Myr BP curves, which are segments of small circles centred on the two Euler poles.

PA-HS motions for the 43-0 Myr BP Euler pole² and for the 10-0 Myr BP pole³ are shown in Fig. 2. Along the transform boundary between the Pacific and North American (PA and NA) plates, if it is assumed that NA-HS motion remained constant as PA-HS motion changed to a more northerly trend, a component of PA-NA motion directed toward the continental margin is added. A compressional tectonics effect is predicted whose magnitude depends on whether, before the change in Pacific plate motion, the plate boundary along this margin was transtensional or transform, which, in turn, depends on the rate of extension in the Basin and Range Province⁴.

Evidence for the predicted plate convergence is present on land along most of the San Andreas fault in the form of intense deformation and uplift that began near the beginning of the Pliocene (~5 Myr BP) and continued into the Quaternary⁵⁻¹². In offshore basins in central California, there is further tectonic evidence for a component of plate convergence across the San

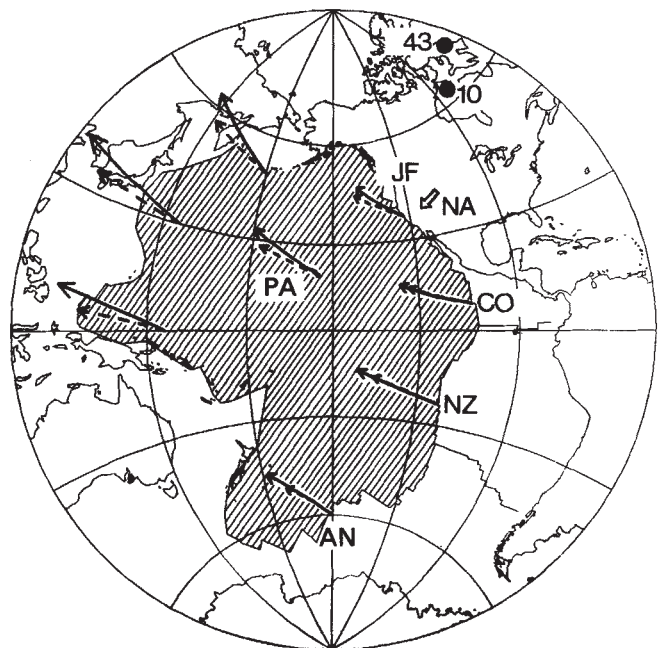


Fig. 2 Velocity vectors of the Pacific plate relative to hotspots. Dashed arrows, motion derived from the 43-0-Myr BP pole² (●). Solid arrows, motion derived from the 10-0-Myr BP pole³ (●). Open arrow, motion of North America relative to hotspots derived from the 10-0-Myr BP pole³.

Juan de Fuca (PA-JF) spreading centre, two major shifts in rotation poles occurred at 8.5 and 5.0 Myr BP¹³, both accompanied by a clockwise rotation in the orientation of PA-JF fracture zones which is predicted by our model if JF-HS motion remained the same before and after the change in PA-HS motion. The magnitude of the observed rotation at 5 Myr BP is about twice that predicted, the difference possibly reflecting a change in JF-HS motion and fragmentation of the Juan de Fuca plate that may or may not¹⁴ have been related to the change in motion of the Pacific plate at 5 Myr BP.

The Pacific-Antarctica (PA-AN) ridge, the longest divergent boundary of the Pacific plate, offers the best opportunity for finding a significant shift in Euler poles at the time of interest. The most accurately determined pre-5 Myr BP Euler poles for PA-AN motion are those of Stock and Molnar¹⁵ for chron 5 (9.8 Myr BP) and chron 6 (19.5 Myr BP), which are distinctly different from the present (that is, 3 Myr BP) pole³ (Fig. 3a). If

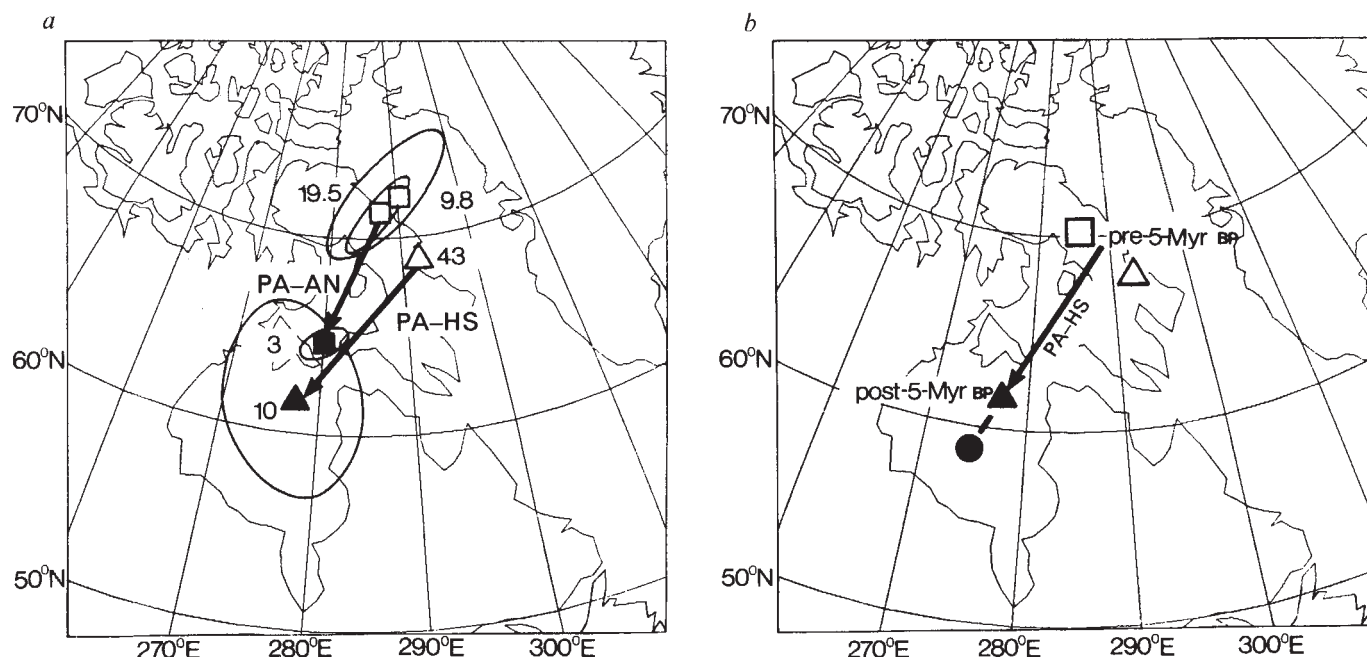


Fig. 3 *a*, Euler poles for restoring Pacific plate to former positions relative to Antarctica plate (■□) and hotspots (▲△). □, PA-AN reconstruction poles¹⁵ for 9.8 and 19.5 Myr BP; ellipses, subjective estimates¹⁵ of regions of acceptable fit for previous poles. ■, PA-AN 3–0 Myr BP pole³. △, PA-HS reconstruction pole² for 43 Myr BP. ▲, PA-HS 10–0 Myr BP pole³; ellipse, standard error³ of previous pole. *b*, PA-HS stage poles for time intervals immediately before and after 5 Myr BP. □, PA-HS pole for 19.5–5 Myr BP determined from PA-AN boundary (Table 2). △, PA-HS pole for 28–5 Myr BP determined from hotspots on Pacific plate (Table 2). ▲, PA-HS pole³ for 10–0 Myr BP determined from hotspots on Pacific plate. ●, Possible 5–0 Myr BP PA-HS pole (59°N, 274°E) based on the assumption that data used for determining the previous pole includes hotspot tracks older than 5 Myr BP.

AN-HS motion did not change at 5 Myr BP whereas PA-HS motion did change, then the expected pattern is one of sub-parallel shifts of the PA-AN and the PA-HS angular velocity vectors before and after 5 Myr BP. Although the Euler poles shown in Fig. 3*a* are finite rotations and not angular velocity vectors, the sense of the shift is clearly that expected if this hypothesis is true.

An independent set of PA-HS poles calculated from the PA-AN-HS circuit provides a more definitive test of the hypothesis. Although AN-HS motion has not been measured directly, it is reasonable to assume that during the Neogene, when the Antarctica plate was bounded mainly by a set of ridges, as it is today, the velocity was low. Therefore, in calculating PA-HS total reconstruction poles from the PA-AN-HS circuit (Table 1), the assumption was that AN-HS motion continued at its present slow rate ($0.054^\circ \text{ Myr}^{-1}$) from 19.5 Myr BP to the present. Using these results, two independent PA-HS stage poles for the time intervals immediately before and after 5 Myr BP were found, one from hotspot tracks on the Pacific plate and the other from the PA-AN-HS circuit. This assumed that PA-AN and PA-HS motion changed at 5 Myr BP. Poles A and B for 5–0 Myr BP (Table 2), determined from the PA-AN-HS circuit and from Pacific hotspot tracks, are almost identical because of the low rate of AN-HS motion. Pole C for 19.5–5 Myr BP was found from the PA-AN-HS circuit assuming that it did not shift during this time. (We chose the chron 6 pole

rather than the chron 5 pole because the former has a much smaller confidence interval¹⁵, the latter gives a rotation pole at a similar location but a larger mean rate of rotation.) Pole D for 28–5 Myr BP was found by starting with coordinates of the 43–0 Myr BP pole² for PA-HS motion, making the assumption that the Euler pole remained fixed from 43–5 Myr BP but that the angular rate of rotation changed at 28 Myr BP¹⁶, and then subtracting from this 28–0 Myr BP Euler pole the 5–0 Myr BP pole (B in Table 2) for PA-HS motion.

In the absence of error, the pre-5-Myr BP poles shown in Fig. 3*b* for PA-HS motion found from the PA-AN-HS circuit and those from hotspots on the Pacific plate would be the same; indeed they agree within the experimental uncertainties. In the observed shift of PA-AN Euler poles is thus consistent with the proposed change in PA-HS motion.

Along the divergent boundary between the Pacific plate and the Nazca¹⁷ (NZ) and Cocos¹⁸ (CO) plates, the predicted changes are small (Fig. 2) and, if present, are not detectable in the presence of ridge jumping and other tectonic noise.

On balance, the observations along the boundary of the Pacific plate are consistent with a clockwise change in the direction of plate motion at ~5 Myr BP possibly preceded by a smaller change at ~8.5 Myr BP. The onset of compressional tectonics at ~5 Myr BP along the San Andreas fault appears to have been rapid, as was the change in PA-JF motion at about the same time, both observations suggesting that a substantial change in

Table 1 PA-HS reconstruction poles obtained by combining AN-HS and PA-AN poles

Age of reconstruction (Myr BP)	AN-HS			PA-AN			PA-HS		
	N. Lat.°	E. Long.°	Angle	N. Lat.°	E. Long.°	Angle	N. Lat.°	E. Long.°	Angle
5	–21.85	255.55	0.27°	64.67	279.77	4.82°	61.84	277.47	4.83°
9.8	–21.85	255.55	0.53°	72.0	290.0	9.75°	69.2	286.0	9.7°
19.5	–21.85	255.55	1.05°	71.25	286.81	15.41°	67.55	283.08	15.34°

Sign convention: the PA-HS poles rotate the Pacific plate back to its position relative to hotspots at the ages shown in the first column, positive angles indicating anti-clockwise rotation. 5 Myr: 3–0 Myr BP PA-AN motion³ extrapolated back to 5 Myr BP; 9.8 Myr BP: chron 5 PA-AN pole¹⁵; 19.5 Myr BP: chron 6 PA-AN pole¹⁵.

Table 2 Stage poles for Pacific-hotspot motion

Pole	Age of reconstruction (Myr BP)		PA-HS pole		Angle	Rate (deg Myr ⁻¹)
	From	To	N. Lat.°	E. Long.°		
A	5	0	61.6	277.5	-4.83°	0.97
B	5	0	61.7	277.2	-4.84°	0.97
C	19.5	5	70.3	285.6	-10.55°	0.73
D	28	5	68.0	292.3	-18.0°	0.78

Sign convention: the rotation is that of the Pacific plate relative to hotspots going forward in time, negative finite angles indicating clockwise rotation about the pole shown (right-hand rule). A, based on PA-AN motion (Table 1), B, based on 10-0 Myr BP PA-HS motion³; C, based on PA-AN motion (Table 1); D, based on PA-HS poles for 28-0 Myr BP (ref. 16) and 5-0 Myr BP (ref. 3).

motion took place within 1 Myr or less. Along the San Andreas fault, compression associated with plate convergence appears to have increased throughout the Pliocene and Pleistocene, which suggests that the clockwise change in the direction of PA-HS motion consisted of two parts: an initial rapid change followed by a slower change in the same sense.

The cause of this change in motion may be analogous to that of the much larger change at 43 Myr BP from northerly (Emperor seamount chain) to westerly (Hawaiian chain) motion, explained¹⁹ as being the result of a change in the location of circum-Pacific subduction zones. Before 43 Myr BP, slabs were attached only along the north and north-west boundary²⁰ and exerted a torque producing PA-HS motion parallel to the Emperor seamount chain¹⁹.

The change in torque required to produce the observed shift in the PA-HS Euler pole at 5 Myr BP could be produced by detaching a subducting slab along the south-west boundary of the Pacific plate. In the mid-Tertiary, a slab subducting to the south was attached to the Pacific plate along most of the boundary between Western Samoa and the Admiralty Islands^{20,21}. Evidence of this palaeosubduction zone includes bathymetric features (for example the Vitiaz trench²²) and island arc geology^{21,23}. In the Solomon Islands the geology indicates that slabs began to detach from the Pacific plate around the middle Miocene (14-11 Myr BP)²¹, possibly causing some of the changes apparent in the central part of the Hawaiian chain. In the New Hebrides arc and Fiji plateau, a southward-subducting slab attached to the Pacific plate was replaced by a northward-subducting slab attached to the Australian plate, either in the middle Miocene²¹ or the early Pliocene²³. The second interpretation is favoured by the observation that the detached slab is still cold and brittle enough to generate deep earthquakes as it sinks beneath the Fiji plateau^{23,24}. Thus, detachment of this slab from the Pacific plate appears to be a probable cause of the change in plate motion at 5 Myr BP.

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Against an aromatic structure for soil fulvic acid

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Soil organic matter is by far the most abundant form of organic matter at the Earth's surface, playing an essential role in soil fertility by maintaining a favourable soil structure, by its water-retention properties and by its ability to store and release fertilizer elements, both cationic and anionic. Yet the nature and origin of its distinctive constituents, the humic substances, remain unknown. These dark-coloured acidic unsaturated compounds are generally considered to be predominantly aromatic and phenolic in character¹⁻³, largely on the basis of the high yields of benzene and hydroxybenzene polycarboxylic acids obtained, using various oxidation procedures, by Schnitzer *et al.*¹. Here we present evidence, at least for the water-soluble fulvic acids, that these aromatic and associated aliphatic oxidation products are possibly, sometimes certainly, artefacts or contaminants introduced by the complex chemical procedures involved in their isolation and identification.

It is difficult to reconcile these oxidation products with ¹³C-NMR (nuclear magnetic resonance) spectra of humic and fulvic acids⁴. The problem is particularly acute with fulvic acid, where ¹³C-NMR indicates that only 35% of the non-carboxylic carbon is unsaturated and that little or none of this is phenolic; its spectrum is more consistent with a proposal, based on analogies between the infrared spectra of fulvic and polymaleic acid⁵, that fulvic acid, when free of associated polysaccharides and peptides, is largely a hydroxyl-substituted unsaturated aliphatic polycarboxylic acid (J. Skjemstad, personal communication). Sharp aromatic features reported in ¹³C-NMR and infrared spectra of a polymaleic acid preparation⁶ are due to pyridinium, introduced during the synthesis (J. D. Russell and H. A. Anderson, personal communication).

Recently, Preston and Schnitzer⁷ have reported that four sharp resonances at 127-131 p.p.m. which may be ascribed to benzene polycarboxylic acids, appear in ¹³C-NMR spectra of humic and fulvic acids treated with diazomethane, with other sharp resonances appearing in the aliphatic region. They suggest that these features are not present in the original fulvic acid because of broadening effects, possibly associated with organic free radicals, metal ions and the presence of hydrogen-bonded micellar regions; they propose that methylation with diazomethane improves resolution by removing adsorbed components and metal impurities and by improving solvation. Of these, a solvation effect can be discounted, given that ¹³C-NMR spectra of solid humic materials, obtained using magic angle spinning, are basically the same as their solution spectra (compare refs 4 and 7); inter- and intra-molecular hydrogen bonding of polycarboxylic acids cannot survive in the alkaline solutions used for NMR examination.

We have tested the other hypotheses by adding 5 mg of each of benzene-1,2,3-tricarboxylic acid and benzene hexacarboxylic acid to an alkaline solution of 120 mg of fulvic acid from the Bh horizon of a Canadian podzol and examining their NMR spectra under the conditions of ref. 7. The added acids produced

sharp resonances, which would have been readily detectable above the broad band of unsaturated carbon in fulvic acid when present as only 1% by weight of fulvic acid. Thus, benzene carboxylic acids, or any similar aromatic system with sharp resonances, would have been readily detected in the original fulvic acids. We conclude that the sharp peaks observed⁷ in diazomethane-treated humic preparations must arise either from aromatic contaminants, or from reaction products of diazomethane with the fulvic acid. Also, there is no good reason to reject the ¹³C-NMR evidence that fulvic acid does not contain all the benzenoid structures which appear in the oxidation products⁴, there being nothing in the nature of fulvate solutions which would obscure their presence. In particular, a suggestion^{1,7} that benzene and hydroxybenzene polycarboxylic acids exist as such in fulvic acid can now be discounted.

As the oxidation products of diazomethane-treated humic materials have been used to infer their structure¹⁻³, we have examined the literature to estimate the contribution of the artefacts or contaminants now shown to be present and to determine whether there may be other contributions to the oxidation products arising from the complex procedures used. There is considerable independent evidence that diazomethane reacts with humic materials to produce byproducts of the desired methylation of hydroxyl groups. Preston and Schnitzer⁷ reported that treatment of humic and fulvic acids with diazomethane caused increases in weight of 20 and 40% respectively. As methylation alone would account for only 8 and 14% respectively, there must be substantial amounts of other reaction products, for example, polymethylene, which has been reported in diazomethane-treated humic acids⁸ and which can be correlated with a peak at 29.7 p.p.m. in ¹³C-NMR spectra of diazomethane-treated humic and fulvic acids⁷. Indeed, polymethylene is a probable source of the straight-chain mono- and di-carboxylic acids that appear among the oxidation products of diazomethane-treated fulvic acid¹.

Diazomethane is highly reactive towards α,β -unsaturated acids with which it can form a great variety of addition and insertion products⁹. Such α,β -unsaturated acids have been shown to constitute a major proportion of the total acidity of a peat humic acid⁸. Reaction of diazomethane with humic acids to form pyrazole derivatives has been established¹⁰; other nitrogen-free reaction products are not improbable. These artefacts cannot, however, be the source of the benzene polycarboxylic acids that appear in the oxidation products of methylated humic materials, as there is no significant difference in yield between methylated and non-methylated humic and fulvic acids^{11,12}. Moreover, reaction of diazomethane with double bonds in humic material is unlikely to create benzene rings of well-defined type, so that the four sharp aromatic resonances mentioned above are more likely to arise from contaminants such as benzenoid plasticizers and anti-oxidants. This is consistent with the absence of the resonances in one fulvic acid preparation and with their uniformity among the other diverse humic and fulvic acids.

Contaminants arising from synthetic polymers and their associated plasticizers and anti-oxidants are difficult to avoid in the laboratory, given the widespread use of plastic vessels and tubing and of polymer-based ion-exchange and chromatographic materials. These contaminants are eliminated by purification procedures in small-molecule organic chemistry, as are unwanted byproducts of reactions. All these substances accumulate in the reaction products of humic materials; for example, the long-chain fatty acids isolated from the Cu₂O/NaOH oxidation and from the NaOH hydrolysis of fulvic acids^{13,14} must be contaminants, as their (CH₂)_n chains would have been detected readily as major features of the ¹³C-NMR spectra of fulvic acid. Infrared spectra of fulvic fractions isolated by gel chromatography show di-alkyl phthalate plasticizer contaminants in amounts which are clearly not present in the starting material (see Fig. 4.8 of ref. 15). We conclude, therefore, that laboratory contaminants are picked up commonly during the manipulation of humic materials and their derivatives.

Given, then, that ¹³C-NMR spectroscopy establishes that fulvic acid does not contain benzenoid structures in sufficient amounts to account for the high yields of benzene and hydroxybenzene polycarboxylic acids in its oxidation products, it remains uncertain whether these arise as artefacts of the oxidation procedures or as contaminants in subsequent manipulations. Martin *et al.*¹⁶ concluded that they were probably artefacts, as they obtained benzene polycarboxylic acids by various oxidations of a polymaleic acid containing little unsaturated carbon. However, they found no phenolic acids among their oxidation products, whereas Spiteller and Schnitzer⁶ did find hydroxybenzene polycarboxylic acids in the oxidation products of polymaleic acid. The difference between the two laboratories suggests a laboratory source of these products.

Whether or not these benzene and hydroxybenzene polycarboxylic acids appear in the oxidation products of fulvic acids, or are introduced in further manipulations, can now be resolved by applying ¹³C-NMR spectroscopy to the unfractionated oxidation products, as they would be readily recognized there if present even as a few % by weight of the starting material. Schnitzer¹ has estimated that they amount to 50% of the starting material, allowing for plausible losses during the complex separations. If they are indeed artefacts of the oxidation procedures and present in such amounts, they are important clues to the structure of fulvic acid.

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Afrotarsius chatrathi, first tarsiiiform primate (? Tarsiidae) from Africa

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Tarsiiform primates have long been regarded as a Laurasian group, with an extensive fossil record in the Eocene of North America and Europe¹⁻⁴ and two important but less well-known records from Asia^{5,6}. The only living genus is *Tarsius* (Tarsiidae), whereas all of the fossil tarsier-like primates are usually placed in the extinct family Omomyidae³. We now report the discovery of *Afrotarsius chatrathi* from early Oligocene rocks of Fayum Province, Egypt. This is the first known tarsiiiform primate from Africa. Compared with fossil primates, the molar tooth morphology of this diminutive prosimian is most similar to that of the European Eocene microchoerine *Pseudoloris*; however, the closest similarity is to the

molars of *Tarsius*. Because the phylogenetic relationships among living *Tarsius* and the omomyids remain unclear^{7,8} and because of the fragmentary nature of the only known specimen of this new primate, allocation of *Afrotarsius* to either Omomyidae or Tarsiidae is necessarily provisional. As we believe that its molar teeth are more like those of *Tarsius* than of any omomyids (including *Pseudoloris*), we tentatively assign the new genus to the extant family Tarsiidae as its only known fossil representative. Recovery of a *Tarsius*-like primate from Africa suggests that it or its ancestors might have been immigrants from Europe, may have been derived from an unknown Asian stock related to the ancestry of *Tarsius*, or may have originated in Africa.

Order Primates
Suborder Prosimii
Infraorder Tarsiiformes
Family Tarsiidae?

Afrotarsius, gen. nov.

Type species: *Afrotarsius chatrathi*, sp. nov.

Diagnosis: Differs from *Tarsius* in having a more posteriorly placed entoconid and thereby relatively longer distance between the M_{1-2} entoconid and metaconid; in having a relatively larger and slightly more labial M_2 paraconid; in lacking a distinct entoconid and in having a smaller, less posteriorly extended posterior cusp on M_3 ; and in having M_3 with a shorter talonid and a relatively smaller crown with respect to M_2 . The specimen differs from all anaptomorphine and omomyine omomyids in the combination of labiolingually broad and shelf-like molar paraconids separated from metaconids and protoconids by a deep notch, and from all omomyids in having a raised, wall-like ridge between the entoconid and metaconid (both features as in *Tarsius*). It differs from all omomyids (except possibly *Hemiacodon* and *Macrotrarsius*) in having M_1 metaconid lingually opposite to protoconid, not placed more posteriorly, and in having a smooth posterior wall on the M_1 trigonid. It differs from all omomyids and *Tarsius* in having an indistinct talonid notch on molars and in having $M_1 > M_2 > M_3$.

Afrotarsius chatrathi, sp. nov.

Etymology: For Prithijit S. Chatrath, collector of the type and only known specimen.

Holotype: CGM (Cairo Geological Museum, Ma'adi, Cairo) 42830, fragment of right mandibular ramus with M_{1-3} , lower parts of crowns of P_3 and P_4 (Figs 1, 2).

Locality: Fossil vertebrate quarry M, 249-m level of Jebel Qatrani Formation (Oligocene), Fayum Province, Egypt. Older than 31.0 ± 1.0 Myr⁹.

Diagnosis: Only known species; same as for genus. Measurements (mm) are: P_4 - M_3 (in series trigonids overlap talonids), 8.70; P_4 length, 1.90; P_4 width, 1.60; M_1 length, 2.45; M_1 trigonid width, 2.00; M_1 talonid width, 2.10; M_2 length, 2.30; M_2 length, 2.20; M_3 trigonid width, 1.80; M_3 talonid width, 2.65; depth of horizontal ramus beneath anterior root of M_2 (lingual side), 3.25.

Description: CGM 42830 is a right lower jaw fragment preserving parts of P_3 - M_3 (Figs 1, 2). The top of the crown of P_3 and most of P_4 are missing, as is much of the labial margin of M_2 . In addition, the protoconid of M_1 and the metaconids of M_1 and M_3 are broken. Anterior to P_3 , part of the distal border of an alveolus is preserved. The lower jaw is slender and shallow and maintains a fairly even depth of 3.25 mm beneath M_{1-3} , shallowing to about 2.90 mm beneath P_3 . A tiny mental foramen is present about 1.20 mm above the inferior border of the jaw and slightly anterior to the anterior root of P_4 .

P_{3-4} are two-rooted teeth, P_3 being the smaller. Both teeth seem to have been essentially unicuspid. A small cristid connects the base of the P_4 protoconid with the tiny hypoconulid on the posterior margin of the tooth. A well-developed labial cingulid becomes confluent posteriorly with this raised distal heel.

In area and length of the molar crowns, $M_1 > M_2 > M_3$. The molars are simple tribosphenic teeth, each with a large, shelf-like paraconid separated from the metaconid and protoconid by a

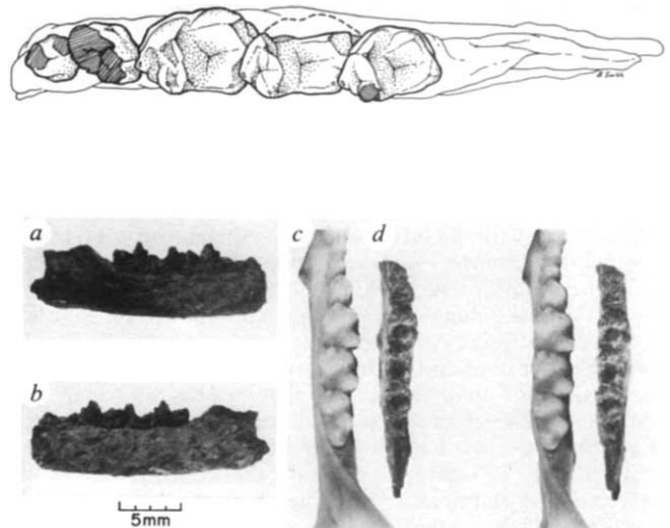


Fig. 2 Labial (a), lingual (b) and occlusal (d) aspects of CGM 42830, holotype of *Afrotarsius chatrathi*, and occlusal aspect (c) of FMNH 57281, *Tarsius syrichta*; c and d are stereo photographs.

deep, curved, posteriorly convex sulcus. On M_{1-2} the paraconids are lingual to the midline mesiodistal axis of the crown and project mesially, giving the trigonid an almost equilateral triangular shape in occlusal view. On M_3 the paraconid is more lingual in position and is slightly closer to the metaconid than on M_1 or M_2 . The M_{1-2} talonids are broad, deeply basined, bounded by hypoconids and entoconids of approximately equal size, and closed posteriorly by small but distinct hypoconulids. The entoconids are located on the distal border of the talonids, with the result that the talonid basins are long mediolaterally. The high wall connecting the entoconids with the bases of the metaconids has an even crest, causing the talonid notch to be indistinct.

M_3 has a somewhat narrower talonid than M_2 , caused by the sharp posterolabial inflection of the entocristid, and possesses only two talonid cusps, a large hypoconid at the posterolabial margin of the crown, and a second cusp (hypoconulid or entoconid) at the centre of the posterior margin of the crown. A high oblique wall connects the hypoconulid with the base of the metaconid. As on M_{1-2} , there is no distinct talonid notch. The M_3 post-hypocristid reaches distally into a sharp notch at the base of the hypoconulid.

The M_{1-3} hypoflexids are shallow, the cristids obliquae reaching the bases of the trigonids slightly lingual to the protoconid. M_1 and M_3 have strong labial cingulids that cross the hypoflexid and merge with strong precingulids and weaker post-cingulids (this part of the crown is missing on M_2).

Afrotarsius chatrathi shows a mosaic of dental similarities to the late Eocene microchoerine *Pseudoloris* and to living South-East Asian *Tarsius*. Traditionally, *Tarsius* has been considered to be a living remnant of some early lineage of omomyid primates, which were diverse and abundant during the Eocene in North America and Europe. More recently, several authors have suggested that *Tarsius* has a closer phyletic relationship with living and fossil higher primates than with omomyids¹⁰⁻¹². Nonetheless, the morphology of the teeth of *Afrotarsius* is clearly closest to that of living *Tarsius* and, among fossil forms, the Omomyidae. We therefore believe, in the absence of other evidence, that the affinities of *Afrotarsius* lie with these animals.

Development of a linguolabially broad and shelf-like molar paraconid separated from the other cusps of the trigonid by a deep valley is a feature shared among *Tarsius*, *Afrotarsius* and *Pseudoloris*, although the more lingual placement of a relatively large paraconid in *Afrotarsius* is more reminiscent of the condition in *Tarsius* than of that in *Pseudoloris*. Also shared is the distinctive posterolabial inflection of the M_3 entocristid, an

unusual feature that seems to link *Afrotarsius* and *Tarsius* to the exclusion of other primate species. The presence of a tall, wall-like entocristid with an indistinct talonid notch, the direct opposition of the M_1 metaconid and protoconid, and the development of tall hypoconids (not relatively short as in *Pseudoloris*) are additional features linking *Afrotarsius* more closely to *Tarsius* than to *Pseudoloris*. Thus, the combination of these and the other diagnostic features demonstrate that *Afrotarsius* is closer in its dental morphology to *Tarsius* than to the Omomyidae, but within that family, *Afrotarsius* most closely resembles *Pseudoloris*.

Unfortunately, canines and incisors, which most clearly distinguish microchoerines and *Tarsius*, are unknown for *Afrotarsius*. Microchoerines, like other omomyids, tend to have a relatively large front tooth in the lower jaw. Of the two teeth immediately posterior to the front tooth, at least one is also relatively large. The anterior three teeth in the lower jaw of *Tarsius*, on the other hand, consist of a very large tooth flanked both anteriorly and posteriorly by much smaller teeth. The divergent structure and placement of the paraconid serve to distinguish the molars of representatives of the omomyid sub-families Omomyinae, Anaptomorphinae and Microchoerinae^{1,13-15}. We believe that the paraconid condition in *Afrotarsius* is most similar to that of *Tarsius* and that in both it is different from that of omomyids. Because only one (if any) of the four basic types of paraconid development can be primitive for primates of modern aspect, we feel that the paraconid condition in *Afrotarsius* is probably the most useful morphological guide to its relative kinship to other primates.

Concerning the palaeobiogeography of Tarsiiformes, relatively little more can be adduced from the discovery of a tarsier-like primate in Egypt. Given the Oligocene palaeogeography of Africa, Europe or Asia are the obvious contenders for the geographical origin of *Afrotarsius* and/or its ancestors. Either solution implies the presence on one of these continents of an unknown stock of *Tarsius*-like Prosimii. Of these two possibilities, an Asian origin is perhaps the more likely, though supported only by circumstantial evidence: (1) there are no known suitable morphological candidates for the ancestry of *Afrotarsius* in the relatively well-sampled fossil record of the Euramerican Eocene Omomyidae; (2) the dentition of *Afrotarsius* is structurally most similar to that of living South-East Asian *Tarsius*; and (3) at least some floral¹⁶ and faunal¹⁷ elements of the Egyptian Oligocene might have been more closely linked to those of various parts of Eocene and present-day South-East Asia than they are to floras and faunas of the early Tertiary of Europe. If *Afrotarsius* or its ancestors immigrated to Africa from either Europe or Asia, this dispersal is most likely to have taken place during the late Eocene-early Oligocene Tethyan regression, an event that facilitated the entry of marsupials into Africa from Europe¹⁸. A last possibility is that *Afrotarsius* originated in Africa from an otherwise unknown (possibly omomyid) prosimian stock. By this viewpoint, *Tarsius*-like primates were deployed from Africa to Asia some time in the Tertiary. However, it is impossible at present to distinguish between these possibilities.

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A variant of the mammalian somatotopic map in a bat

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Two ordered representations of the body surface, S-I and S-II, have been described on the cortical surface of the brains of a variety of mammals; additional separate topographical maps have been found in the somatosensory cortex of the cat and monkey¹⁻⁵. Except for minor variations in the placement of the body parts, the basic somatotopy of the maps is remarkably consistent across species⁵. As the reasons for this consistency and the minor variations are unclear, we examined the somatotopy of the bat, whose body plan has been modified extensively so that the forelimb can be used for flight⁶. We report here that in both S-I and S-II of the grey-headed flying fox, not only is the representation of the distal forelimb displaced from its usual position on the map, but the digits are directed caudally instead of rostrally as they are in all other mammals studied. The variant somatotopy appears to reflect the postural differences between flying and walking mammals, supporting the notion that topographical maps may have functional significance apart from their point-to-point connections with the sensory periphery.

Standard microelectrode mapping techniques (see, for example, ref. 4) were used to study the organization of

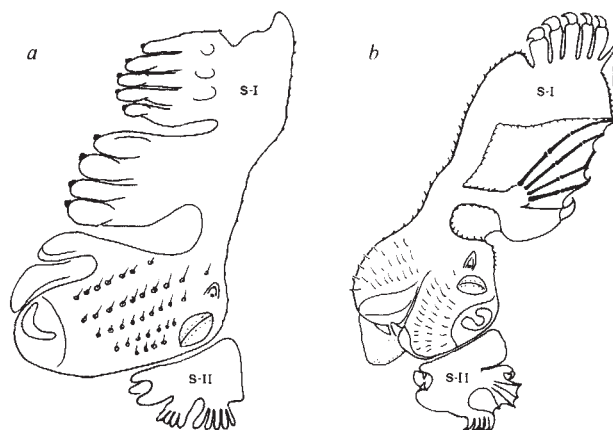


Fig. 1 Schematic representations of the body surface ('homunculi') on the somatosensory cortex of the rat (*a*, redrawn from ref. 5) and the flying fox (*b*, drawn, with artistic licence, from data on 785 recording sites and summarized more accurately in Fig. 2*d*). The rat, like opossum, squirrel, galago, cat, tree shrew and various New World and Old World monkeys (see ref. 5) has a somatotopic representation in which the forelimb digits are directed rostrally in both S-I and S-II. In contrast, the bat has a representation in which the forelimb digits are directed caudally, reflecting the altered position of these digits for use in the wing.

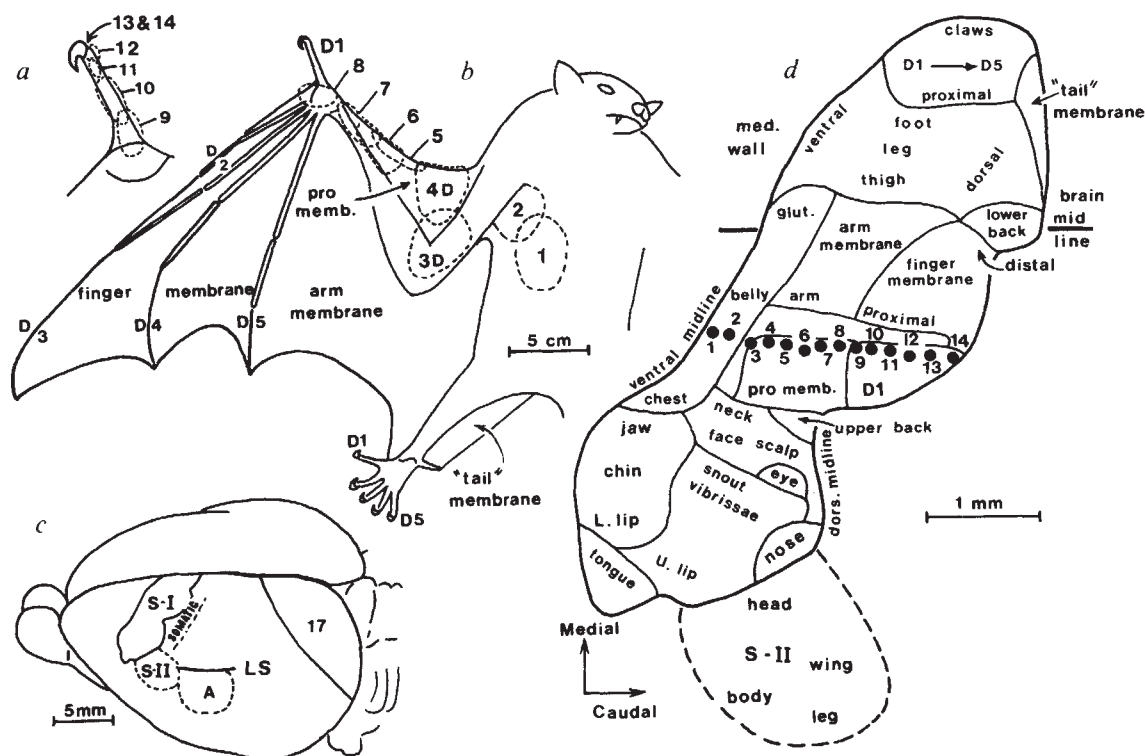


Fig. 2 Organization of somatosensory cortex in the flying fox. Receptive fields for a rostrocaudal row of recording sites in S-I (see *d*) are outlined on ventral views of the body (*b*) and first digit of the wing (*a*). Receptive fields 3D and 4D, shown on the ventral surface, were actually in the corresponding location on the dorsal surface of the arm and pro-wing membrane. Receptive fields 13 and 14 were at the base of the claw of the first digit (D1). *c*, A dorsolateral view of the brain with S-I, S-II and a third somatosensory region indicated. A, auditory cortex; area 17 is primary visual cortex; LS, lateral sulcus. *d*, Detailed organization of S-I and S-II and the locations of electrode penetrations which yielded the receptive fields shown in *a* and *b*. In this experiment boundaries were determined from receptive field locations for 386 other recording sites not shown. Note that the most medial portion of S-I, containing the representation of the hindlimb and tail membrane, extends down onto the medial wall of the brain where it is not visible in the dorsolateral view (*c*). This part of the map (medial to the line marked 'brain midline') has been folded out 'flat' for illustration purposes.

somatosensory cortex in a Megachiropteran, frugivorous, non-echolocating bat, *Pteropus poliocephalus*, the grey-headed flying fox. Five animals were anaesthetized with a combination of ketamine hydrochloride (20 mg per kg intramuscular, i.m.) and xylazine (1 mg per kg i.m.), supplemented with pentobarbitone (6 mg per kg) in some individuals. Light tactile stimuli defined receptive fields on the body surface for single units and clusters of units at 785 recording sites in the somatosensory cortex. Recording sites, projected orthogonally to the cortical surface, were grouped according to receptive field location so that sites with receptive fields located primarily or exclusively on a particular body part (such as the jaw, or pro-wing membrane; see Fig. 2) were outlined. In this manner, the arrangement of body parts in the cortical representation became apparent. In addition, the auditory cortex and its boundary with the somatosensory cortex was mapped with tones in two bats, and the cortical projection areas of primary visual cortex determined with anatomical tracing techniques in several other animals.

We found three separate representations of the body surface in somatosensory cortex in addition to multiple representations in the auditory and visual cortices. The microelectrode mapping results indicate that the arrangement of body parts in S-I and S-II of bats is substantially different from the usual mammalian scheme. In other mammals, the organization of S-I can be crudely summarized as a 'homunculus' or distorted representation of the body surface with the head lateral, the tail medial, and the digits of the hands and feet rostral as in Fig. 1*a*. Distortions due to differential enlargement of body parts are apparent, and discontinuities in the representation occur⁵, but the basic orientation of the forelimb digits towards the rostral border of S-I remains consistent. S-II adjoins S-I along the representation of the dorsal midline of the head, and S-II can

be regarded as a smaller, less detailed mirror image of S-I. Thus, if mental allowances are made for the rotation of S-II, and its bending into fissures in some species, the distal digits and face all point 'rostrally' in both S-I and S-II.

A quite different picture emerges when the detailed maps of S-I and S-II of the flying fox are considered (Fig. 2*d*). When the results are summarized as a homunculus (Fig. 1*b*), the orientation and placement of the face and hindlimb remain basically the same as for other mammals. However, the relative placement of the forelimb is reversed so that the distal digits extend caudally in both cortical areas. The prominent claw of the thumb is at the caudal border of S-I, in contrast to its position at the rostral border of S-I in other mammals. Moreover, the ventral trunk under the wing is represented rostral to the wing, unlike its typical representation in other mammals. The dorsal trunk is sparsely represented, and in S-I the caudal and rostral portions are split into caudomedial and caudolateral cortical locations (Fig. 2*d*). Note that the usual mammalian position of the forelimb in S-I and S-II, relative to the other body parts, corresponds to the actual relationship of the forelimb to most other body parts in standing and feeding, while the relative position of the distal forelimb in the somatosensory representation of the bat corresponds more closely to the postural relationship of the wing to other body parts.

Figure 2 shows an example of the electrophysiological evidence for the caudal location of the distal forelimb in S-I. S-I and S-II are in their usual locations relative to auditory and visual fields in the forebrain (Fig. 2*c*). Figure 2*d* summarizes the organization of S-I and S-II, showing the positions in the two representations at which microelectrodes encountered neurones with receptive fields located in the designated body parts. A row of recording sites across S-I produced a progression

in receptive field position from the ventral trunk to the wing of the arm and then distally to the wing of the metacarpal bones and fingers. These recording sites represent a small fraction of the 400 sites that were used to determine the organization of S-I in this animal. These data and those from the other bats are consistent with the conclusion that the distal forelimb representation of the bat is shifted caudally in S-I and S-II, compared with other mammals.

Apart from alteration in the representation of the trunk, which may be related to the unusual location of the forelimb representation, other parts of S-I and S-II correspond to the basic mammalian pattern. As in other mammals, differing amounts of cortex are related to different body parts. For example, a considerable amount of cortex is devoted to the hairs of the face, which are important sensory organs in bats and most other mammals. The free first digit of the wing is well represented, as is the anterior wing or pro-wing (the part of the wing that would most often encounter objects). The representation of the forelimb and associated wing membrane constitutes over one-fifth of S-I, a proportion similar to that found in Microchiropteran bats⁷.

In common with many other mammals, bats have several sensory representations in the cortex. Besides S-I and S-II, we obtained clear evidence for a third somatosensory representation caudal to S-I. The organization of this field was not fully determined because it was not reliably driven, and it appeared to be quite sensitive to anaesthesia. The auditory cortex contained at least two representations of tone frequency. Anatomically revealed projections from the primary visual cortex, V-I, indicated at least two additional visual areas: V-II and a visual area in more temporal cortex. Our finding of multiple representations of the senses in the cortex of a Megachiropteran bat follows a wealth of data on multiple representations in the cortex of advanced mammals⁸; analogies with these studies suggest that the bat has a moderately advanced brain.

We have shown that the placement of the distal forelimb is altered in S-I and S-II of bats; this may help to elucidate the constraints that determine the location and orientation of body parts in somatotopic maps. In view of the recent evidence that the nervous system of the owl has a 'computational' map of auditory space which can be obtained only indirectly from the sensory periphery, as the latter is tonotopic but not spatiotopic⁹, it seems possible that one such constraint is the need to maintain an appropriate relationship between different brain maps of extrapersonal space. If the relationship between the somatotopic map and other brain maps of extrapersonal space has been constrained during phylogeny or ontogeny to depend on the habitual spatial orientation of the animal's body, this may explain the reversed representation of the bat's limbs and digits, as these are caudal to the head during flight or above it during rest, in contrast to most other mammals in which the forelimb is used under, or in front of, the head.

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An *N*-methyiaspartate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine?

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It has been proposed that three major receptor subtypes subserve the putative transmitter role of glutamate and aspartate in the mammalian central nervous system. One subtype is classified by the specific agonist *N*-methyiaspartate (NMA)^{1–4} and the specific antagonist 4-amino-2-phosphonovaleic acid⁴. It has been shown recently that excitation of neurones by NMA is also selectively reduced by dissociative anaesthetics such as ketamine and phencyclidine^{5,6} and by sigma opiates⁷, drugs of abuse with common psychotomimetic properties^{8,9}. Responses to NMA have an unusual voltage relation^{10,11} which may result from a voltage-dependent block of the activated channel by physiological concentrations of magnesium^{12–14}. No synaptic potential with properties similar to those of responses to NMA, however, has yet been reported. We describe here an excitatory postsynaptic potential (e.p.s.p.) evoked by electrical stimulation of the white matter and recorded intracellularly from pyramidal cells in slices of rat somatosensory cortex. This e.p.s.p. has the appropriate voltage relation and sensitivity to Mg²⁺ and ketamine to be an NMA receptor-mediated synapse and a potential central site for the psychotomimetic actions of ketamine.

Recordings were first obtained from coronal slices of cortex, maintained in standard medium containing 1 mM Mg²⁺. Cells ($n = 37$) were selected according to the following criteria. They maintained resting membrane potentials (RPs) > -75 mV, action potentials > 70 mV with overshoots of 10–30 mV and thresholds 15–30 mV positive to RP, and input resistances apparently larger when measured with depolarizing than with hyperpolarizing pulses. Depolarizing pulses evoked slow depolarizations that elicited fast spikes. These properties are similar to those reported previously for cortical neurones *in vitro*^{15–18}. The cells could be activated antidromically from the corpus callosum, indicating that they were pyramidal cells. Full somatic invasion by the antidromic spike was, however, achieved only when the cells were depolarized by 20–30 mV. All recorded cells were 100–300 μ m from the pial surface.

Electrical stimulation of the ipsilateral callosum with low stimulus strengths (0.2 ms; < 10 V) evoked a small, long-latency e.p.s.p. whose voltage relation differed from those of p.s.ps reported previously in that it increased in both amplitude and duration as the membrane was depolarized and decreased as the membrane was hyperpolarized from RP (Fig. 1). In 16 cells, it was possible to evoke such an e.p.s.p. alone; in others, it appeared as a component of a more complex postsynaptic response. Repetitive stimulation (1–2 Hz) of the callosum resulted either in no change in the amplitude of the low-threshold e.p.s.p., or in a small diminution. As described previously^{19,20}, stimulation of the callosum with higher stimulus strengths (0.2 ms; < 30 V) led to a complex series of p.s.ps, lasting > 100 ms. Typically, a large, short-latency e.p.s.p., which decreased in amplitude as the membrane was depolarized, was followed by one or more inhibitory potentials that were negative-going at membrane potentials 10–20 mV positive to RP.

All cells tested ($n = 15$) depolarized in response to pulses of NMA (1–4 s, 40–100 nA). These responses decreased in amplitude with hyperpolarization (Fig. 1), as observed in studies of the responses of other neurones to NMA^{10,11}. In contrast, responses to glutamate showed a more conventional voltage relation (Fig. 1).

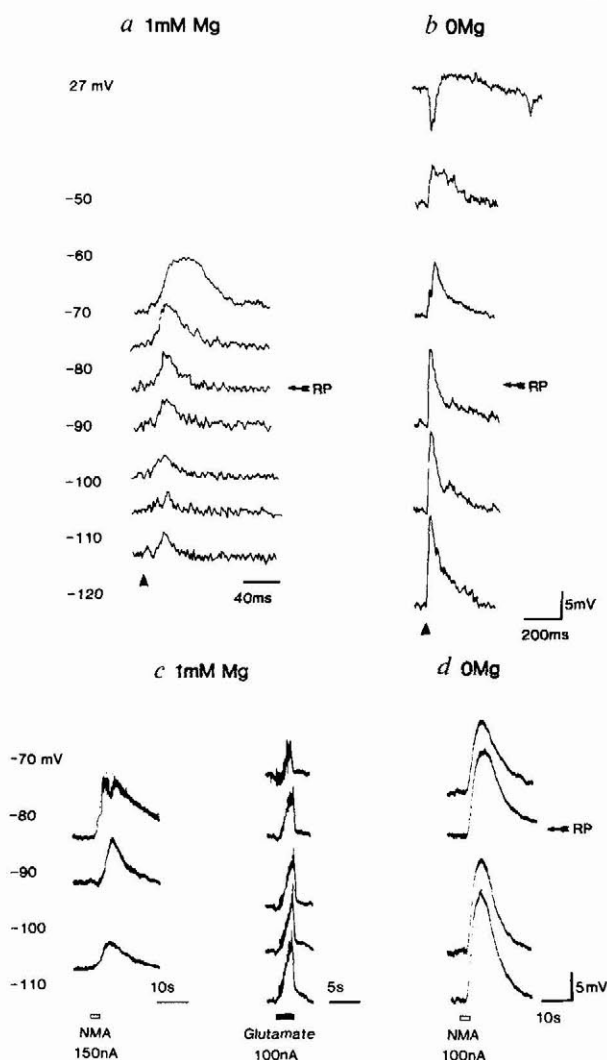
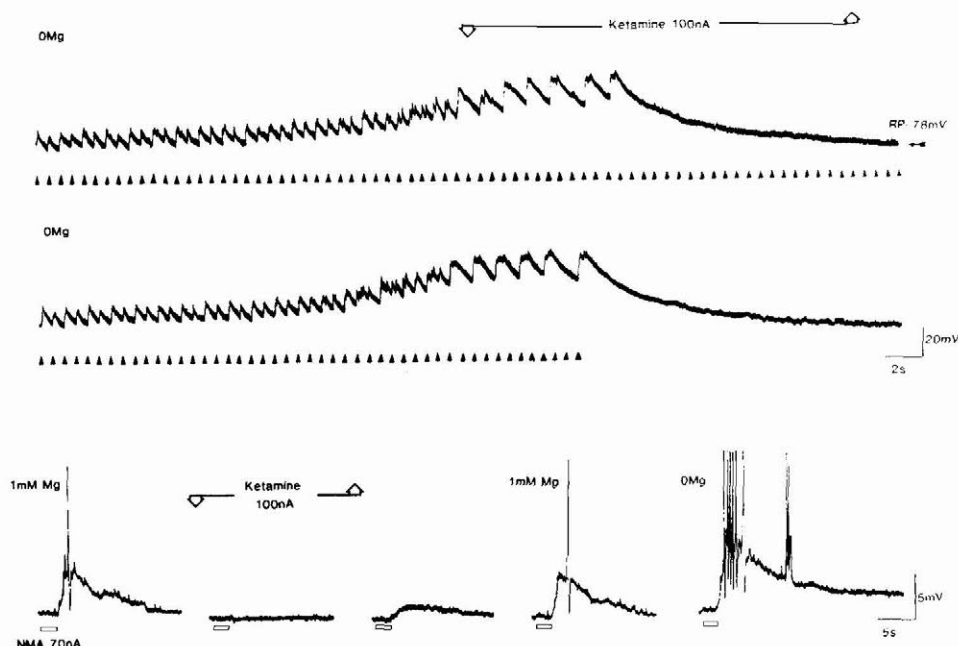


Fig. 1 Removal of extracellular Mg^{2+} changes the voltage relations of the low-threshold e.p.s.p. and responses to NMA. In the presence of 1 mM Mg^{2+} , low-threshold e.p.s.ps evoked in cortical neurones by stimulation of the corpus callosum ($\blacktriangle$) (a) and responses to iontophoretic pulses of NMA ($\square$) (c) decrease in amplitude with membrane hyperpolarization. In contrast, in the absence of Mg^{2+} , low-threshold e.p.s.ps (b) and NMA responses (d) increase with hyperpolarization. Responses to glutamate increase with hyperpolarization even in the presence of Mg^{2+} (c). Recordings in b, c and d are from the same neurone. Note the different time scales in a and b and for responses to NMA and glutamate in c. In d the NMA current is smaller, but the response is larger than in c.

Methods. Intracellular recordings were obtained from coronal slices of somatosensory cortex 400 μ m thick, from young adult male rats ($n = 15$). Slices were cut using a Vibroslice, transferred to the slice chamber (modified from ref. 24) and maintained at 35 $^{\circ}$ C at the interface between artificial cerebrospinal fluid (124 mM NaCl, 25.5 mM $NaHCO_3$, 3.3 mM KCl, 1.2 mM KH_2PO_4 , 2.5 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM D-glucose) and warm humidified gas (95% O_2 /5% CO_2). Flow rates were about 0.2 ml min^{-1} , bath volume 0.2 ml. Recording electrodes were 3 M K-acetate-filled glass micropipettes of tip resistance 60–120 M Ω . A preamplifier with conventional bridge balance and current injection facilities allowed the membrane potential to be changed. The corpus callosum was stimulated using a bipolar electrode made from twisted 50- μ m insulated Ni/Cr wire. N-methyl-D,L-aspartate (NMA, 200 mM, pH 8), ketamine (50 mM in 150 mM NaCl, pH 4) and glutamic acid (200 mM, pH 8) were ejected from a multi-barrelled pipette which also included a balance barrel (150 mM NaCl). This pipette was placed close to the tip of the recording electrode and positioned to give the best response of the recorded cell to NMA.

Fig. 2 Ketamine-induced blockade of both the low-threshold e.p.s.p. and responses to NMA. Intracellular recordings from a cortical neurone. Top record, repetitive stimulation of the corpus callosum ($\blacktriangle$) in the absence of extracellular Mg^{2+} leads to potentiation of the e.p.s.p. and a plateau depolarization. A 100-nA current of ketamine blocks the response completely, despite continued stimulation, and the cell slowly repolarizes. Middle record, continuation of the top record after 3 min continuous stimulation, showing reversal of ketamine effects, potentiation of the e.p.s.p. and, on cessation of the stimulation, slow repolarization of the cell. Bottom records, left, response to a 2-s, 70-nA ejection of NMA ($\square$) is totally blocked by a 30-s ejection of ketamine, 100 nA (second record). After 4 min (third record) partial reversal of the ketamine effect is seen and reversal is complete after 5 min (fourth record). Final record shows that removal of extracellular Mg^{2+} leads to an increase in the response of this cell to NMA.



As Mg^{2+} modifies the action of NMA¹²⁻¹⁴, these tests were repeated in Mg^{2+} -free medium. Within 25–60 min of the changeover, low-threshold e.p.s.ps were measurably larger in amplitude and duration. Repetitive stimulation resulted in a further potentiation of this response, a depolarization plateau (Fig. 2) and, in some cells, bursts of slow potentials, ~20 mV in amplitude and 40 ms in duration, each of which evoked a burst of fast spikes. Potentiated e.p.s.ps could still be evoked several minutes after termination of repetitive stimulation. Similarly, depolarizing responses to NMA were increased in amplitude and duration in Mg^{2+} -free medium (Figs 1, 2), so much so that it was usually necessary to decrease the NMA dose to prevent large sustained depolarizations.

The voltage relations of both the low-threshold e.p.s.p. and the response to NMA were also affected by the removal of extracellular Mg^{2+} . These responses now increased in amplitude with hyperpolarization (Fig. 1). All changes induced by the removal of Mg^{2+} were reversed within 10–15 min of its re-introduction. Neither the complex p.s.p., the response to intracellular current injection nor the response to glutamate were substantially affected by the removal of Mg^{2+} .

Ketamine (40–100 nA for 5–30 s) greatly reduced the responses of cortical neurones to NMA (Fig. 2), but not to glutamate, in both 1 mM Mg^{2+} -containing and Mg^{2+} -free medium. The same doses of ketamine also reduced the low-threshold e.p.s.p. and prevented its potentiation. Even established plateau depolarizations were blocked by ketamine (Fig. 2), although high-threshold p.s.ps were unaffected. These effects of ketamine were reversed 2–5 min after terminating its ejection (Fig. 2). In a further five cells, the competitive NMA antagonist 4-amino-2-phosphonovaleric acid also selectively reduced responses to NMA and the low-threshold e.p.s.p.

The similarity between the properties of the low-threshold e.p.s.p. and the depolarization elicited by NMA, both in their unusual voltage relation and in their sensitivity to Mg^{2+} and ketamine, are consistent with a role for NMA receptors in the synaptic activation of cortical neurones. The unusual voltage relation in the presence of Mg^{2+} and the sensitivity of the responses to Mg^{2+} could be explained by the voltage-dependent block by Mg^{2+} of the activated channel proposed by Nowak *et al.*¹³ and Mayer *et al.*¹⁴. At membrane potentials negative to rest, the block would be powerful and responses much reduced. With depolarization, the block would decrease and responses increase in amplitude. On removal of Mg^{2+} , the underlying, conventional voltage relation of the channel would be revealed and responses would increase progressively with hyperpolarization. Although amino acids have been implicated in synaptic transmission^{4,21}, this is the first intracellular study clearly linking NMA receptors and synaptic excitation. Manipulation of membrane potential and Mg^{2+} levels *in vitro* may reveal similar e.p.s.ps in other brain areas.

Reduction of such cortical e.p.s.ps may contribute to the behavioural effects of ketamine and other dissociative anaesthetics, since the effects of NMA are blocked *in vivo* by doses of ketamine below those required for anaesthesia⁵ but similar to those that induce psychotomimetic effects in man^{8,22} and delirium in animals⁹. The potency of dissociative anaesthetics and sigma opiates relative to phencyclidine in behavioural tests correlates well with their potency as selective NMA antagonists²³. Further studies will be required to assess the implications of these results with respect to the psychotomimetic effects of systemically active NMA antagonists.

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A common sequence of calcium and pH signals in the mitogenic stimulation of eukaryotic cells

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When normal quiescent (G_0) cells are stimulated by mitogens to enter the cell cycle, the metabolic derepression which occurs^{1–8} is similar in a variety of cells. The mechanisms initiating these responses and their relationship to subsequent progression through G_1 to DNA synthesis in S phase, however, are generally undefined. The clearest evidence has been obtained in sea urchin eggs⁹, where fertilization by sperm causes a rapid, transient increase in the concentration of free cytoplasmic Ca^{2+} ($[Ca]_i$), followed by a sustained increase in cytoplasmic pH (pH_i). It has been demonstrated clearly that these ionic responses are obligatory for progression to DNA synthesis by the normal pathway after fertilization, although the Ca^{2+} signal can be bypassed by parthenogenetic agents which elevate directly pH_i (for example, NH_4^+ ions)⁹. These observations raise the questions of whether other eukaryotic cells show the same sequence of ionic responses when stimulated by mitogens and whether such signals are an obligatory component of their mitogenic pathways. We show here that a common sequence of $[Ca]_i$ and pH_i responses occurs in both quiescent mouse thymocytes and Swiss 3T3 fibroblasts stimulated by appropriate mitogens. Furthermore, 'opportunistic' mitogens (those that do not act on the cells *in vivo*, such as concanavalin A (Con A)¹⁰, the Ca^{2+} ionophore A23187¹¹ and 12-*o*-tetradecanoyl phorbol 13-acetate (TPA)^{12,13}) that are mitogenic for both mouse thymocytes and 3T3 fibroblasts¹⁴, each produce characteristic ionic responses that are the same in both types of cell.

Previous studies on freshly isolated thymocytes and lymphocytes have shown that a 1.5–2-fold increase in $[Ca]_i$ occurs within 1–2 min of addition of ligands which cross-link mitogen receptors on the cell surface (for example, Con A^{15,16}, phytohaemagglutinin¹⁶, anti-immunoglobulin antibody¹⁷ and the monoclonal antibody UCHT 1, ref. 18). In thymocytes stimulated by mitogenic concentrations of Con A, this Ca^{2+} signal declines slowly over 24 h¹⁹. However, we have noted previously that, in freshly isolated thymocytes, there is a population of cells in S phase which is highly active metabolically, consistent with previous reports for related cell systems²⁰. When the cells are loaded with quin 2 (ref. 21), the fluorescence signal is weighted in favour of the active cells, which are ~10-fold larger in volume than the quiescent cells. We therefore re-

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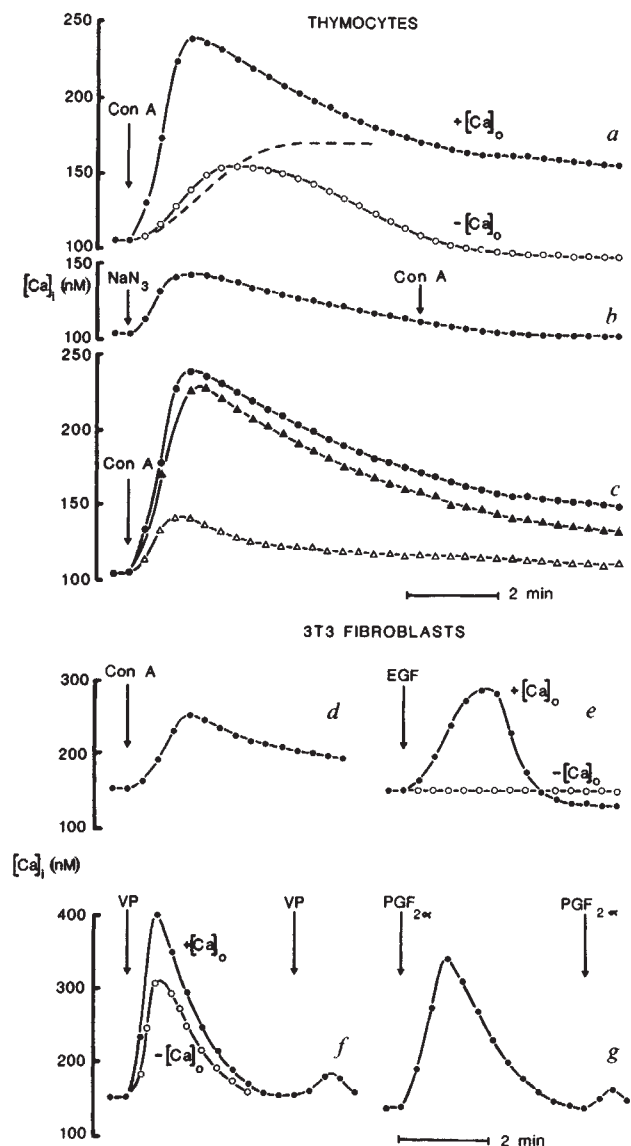


Fig. 1 Changes in free intracellular Ca^{2+} ($[\text{Ca}]_i$) in mouse thymocytes and Swiss 3T3 fibroblasts following the addition of mitogens. *a*, Con A ($1 \mu\text{g ml}^{-1}$) added as indicated to quiescent thymocytes in: $\bullet$, BSS ($[\text{Ca}]_o = 0.43 \text{ mM}$); $\circ$, BSS with 1 mM EGTA ($[\text{Ca}]_o < 1 \mu\text{M}$). The dashed curve shows the corresponding response of freshly isolated thymocytes in BSS. *b*, NaN_3 (10 mM) followed by Con A ($1 \mu\text{g ml}^{-1}$) to quiescent thymocytes in BSS; *c*, $\bullet$, Con A ($1 \mu\text{g ml}^{-1}$) added alone as indicated to quiescent thymocytes in BSS; Δ , Con A ($1 \mu\text{g ml}^{-1}$) added 10 min after TPA (10 nM); $\triangle$, Con A ($1 \mu\text{g ml}^{-1}$) added 10 min after 8-bromo-cyclic AMP (10 mM). *d*, Con A ($2 \mu\text{g ml}^{-1}$) added to 3T3 fibroblasts in medium B. *e*, EGF (12 ng ml^{-1}) added to 3T3 fibroblasts in medium B ($\bullet$) or in medium B with 1.5 mM EGTA ($[\text{Ca}]_o < 1 \mu\text{M}$) ($\circ$). *f*, $\bullet$, Successive additions of VP (10 ng ml^{-1}) to 3T3 fibroblasts in medium B; $\circ$, VP (10 ng ml^{-1}) added to cells in medium B immediately after addition of 1.5 mM EGTA ($[\text{Ca}]_o < 1 \mu\text{M}$). *g*, $\bullet$, Successive additions of $\text{PGF}_{2\alpha}$ (100 ng/ml) to 3T3 fibroblasts in medium B.

Methods. *Cell preparation.* Thymocytes from BALB/c mice were prepared in RPMI 1640 medium buffered with 10 mM Tris, 24 mM NaHCO_3 , pH 7.3, washed twice by centrifugation ($250g$, 3 min) into fresh medium, incubated with continuous stirring at $20 \times 10^6 \text{ ml}^{-1}$ for 16 h at 37°C in air/ CO_2 (19:1) and washed twice by centrifugation into Earle's balanced salts solution buffered with 10 mM HEPES, pH 7.3 (BSS). Swiss 3T3 fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 5% newborn calf serum, 5% fetal calf serum (Gibco), 44 mM NaHCO_3 , 100 U ml^{-1} penicillin, $100 \mu\text{g ml}^{-1}$ streptomycin and $0.25 \mu\text{g ml}^{-1}$ Fungizone (Gibco) (medium A) in a humidified atmosphere of air/ CO_2 (9:1) at 37°C . Cells (6×10^6) were seeded onto 0.4 g Cytodex 1 microcarrier beads (Pharmacia) and grown to confluence in 4–6 days in 200 ml of medium stirred continuously at 50 r.p.m. Before determination of $[\text{Ca}]_i$ and pH_i , samples of the bead culture were incubated for 24 h in serum-free medium A. *Intracellular indicator loading and $[\text{Ca}]_i$ measurement.* Thymocytes ($20 \times 10^6 \text{ ml}^{-1}$) were incubated at 37°C for 45 min in BSS containing $2 \mu\text{M}$ ^3H -quin 2 acetoxymethyl ester (^3H -quin 2-AME, 5.4 Ci mol^{-1} , ref. 15) before washing and suspension at $6 \times 10^6 \text{ ml}^{-1}$ in BSS for fluorescence measurements. Confluent 3T3 cells attached to Cytodex 1 microcarrier beads were allowed to settle under gravity (2 min), washed once in medium B (119 mM NaCl, 5 mM KCl, 25 mM PIPES, 5.6 mM glucose, 1 mM CaCl_2 , 0.4 mM MgCl_2 , 0.1% BSA, pH 7.2) and resuspended in 5 ml of medium A ($\sim 0.02 \text{ g}$ Cytodex 1 beads) containing $10 \mu\text{M}$ ^3H -quin 2-AME. The suspensions were then incubated on a rotary mixer (2 r.p.m.) for 45 min at 37°C , washed twice and suspended in 1.5 ml of medium A for fluorescence measurements in a 1 cm quartz cuvette (3×10^5 cells) at 37°C in a Perkin Elmer 44B spectrofluorimeter with continuous stirring. The hydrolysis of intracellular ^3H -quin 2-AME was confirmed by the shift in emission maximum from 435 nm to 492 nm for quin 2 (excitation wavelength 339 nm) and $[\text{Ca}]_i$ was calibrated and corrected for external quin 2 as described previously for thymocytes¹⁵ and fibroblasts²⁴. The data points in all figures are calculated from the traces of fluorescent intensity, integrated over 5 s .

examined the Ca^{2+} signal in thymocytes allowed to become quiescent over 12–18 h before loading with quin 2 and adding Con A.

The Ca^{2+} signal in the metabolically quiescent cells had a large transient component which rose to $\sim 250 \text{ nM}$ and then declined over 10–15 min to a steady level comparable with the persistent Ca^{2+} signal observed in freshly isolated cells (Fig. 1a). In quiescent cells, immediately after the removal of extracellular Ca^{2+} ($[\text{Ca}]_o < 1 \mu\text{M}$) by the addition of EGTA, a smaller transient Ca^{2+} signal was observed in response to Con A declining back to the initial $[\text{Ca}]_i$ over 6–8 min (Fig. 1a). This transient signal must be derived from Ca^{2+} in an intracellular pool, and is diminished after prolonged exposure to low $[\text{Ca}]_o$. The characteristic features of Ca^{2+} release from an intracellular pool, that is, a transient Ca^{2+} signal followed by rapid return to the initial $[\text{Ca}]_i$ level, were observed also in quiescent thymocytes treated with 10 mM NaN_3 (Fig. 1b), which reduces the cellular ATP level by 75% (ref. 22). No Ca^{2+} signal was observed subsequently in these cells in response to Con A (Fig. 1b, see also ref. 23). The Ca^{2+} signal in metabolically quiescent cells therefore seems to consist of a transient component derived from Ca^{2+} in an intracellular pool and a persistent component which we show elsewhere can be attributed to the inhibition by Con A of Ca^{2+} efflux across the plasma membrane. $12\text{-}o\text{-TPA}$ (10 nM) had little effect on $[\text{Ca}]_i$ in quiescent cells or on the transient Ca^{2+} signal

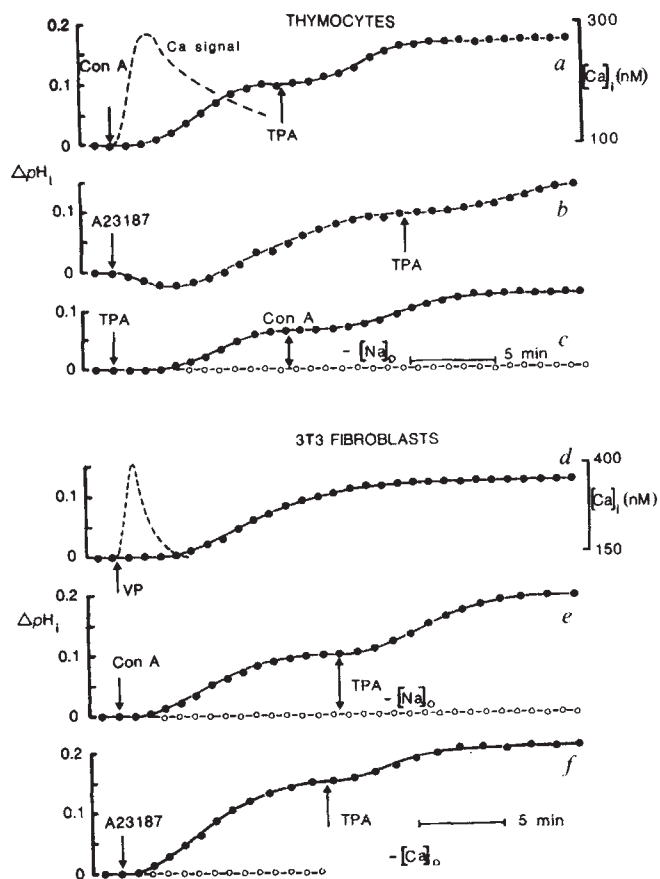
in response to Con A. Addition of 8-bromo-cyclic AMP (10 mM), which inhibits mitogenic stimulation of thymocytes, markedly decreased the transient $[\text{Ca}]_i$ response to Con A (Fig. 1c).

We have shown recently²⁴ that in quiescent Swiss 3T3 fibroblasts loaded with quin 2, some mitogens cause a rapid transient increase in $[\text{Ca}]_i$ (see also refs 25, 26). Figure 1d–f shows the rapid Ca^{2+} signals that follow addition of Con A or of the other fibroblast mitogens epidermal growth factor (EGF), vasopressin (VP) or prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$). These signals were transient and $[\text{Ca}]_i$ usually returned to a stable value $\pm 20\%$ of resting $[\text{Ca}]_i$ ($153 \pm 8 \text{ nM}$, s.e.m.; $n = 25$) within 4 min of addition of mitogen. Repeated additions of a single agent at optimal mitogenic concentrations gave very small Ca^{2+} transients after the first addition. Mitogenic concentrations of TPA (10 nM), 8-bromo-cyclic AMP (10 mM) or insulin ($1 \mu\text{g ml}^{-1}$) did not increase $[\text{Ca}]_i$ over 10 min . TPA (10 nM) abolished the Ca^{2+} signal in response to EGF (Fig. 3c), but had only small effects on the Ca^{2+} signal in response to VP or $\text{PGF}_{2\alpha}$ and higher TPA concentrations ($> 20 \text{ nM}$) were required to antagonize these Ca^{2+} signals. 8-Bromo-cyclic AMP and insulin had no significant effect on the Ca^{2+} signal in response to any mitogen.

Reduction of $[\text{Ca}]_o$ to $< 1 \mu\text{M}$ by the addition of 1 mM EGTA immediately abolished the Ca^{2+} transient in response to EGF (Fig. 1e), although it did not block either the pH_i response (see

Fig. 2 Changes in cytoplasmic pH (pH_i) in mouse thymocytes and Swiss 3T3 fibroblasts following the addition of mitogens. *a*, $\bullet$, Con A ($1 \mu\text{g ml}^{-1}$) and TPA (10 nM) added to quiescent thymocytes in BSS+2.5 mM NaHCO_3 ; the dashed line is the Con A-induced $[\text{Ca}]_i$ response. *b*, $\bullet$, A23187 (100 nM) and TPA (10 nM) added to quiescent thymocytes in BSS. *c*, TPA (10 nM) and Con A ($1 \mu\text{g ml}^{-1}$) added to quiescent thymocytes in BSS ($\bullet$) and in Na^+ -free BSS (containing 120 mM choline chloride and 2.5 mM KHCO_3) ($\circ$). *d*, $\bullet$, VP (10 ng ml^{-1}) added to 3T3 fibroblasts in medium B+2.5 mM NaHCO_3 ; the dashed line is the VP-induced $[\text{Ca}]_i$ response. *e*, Con A ($2 \mu\text{g ml}^{-1}$) and TPA (10 nM) added to 3T3 fibroblasts in medium B+2.5 mM NaHCO_3 ($\bullet$) and in Na^+ -free medium (medium B containing 119 mM choline chloride and 2.5 mM KHCO_3) ($\circ$). *f*, $\bullet$, A23187 (100 nM) and TPA (10 nM) added to 3T3 fibroblasts in medium B; $\circ$, A23187 (100 nM) added to cells in medium B with 1.5 mM EGTA ($[\text{Ca}]_o < 1 \mu\text{M}$).

Methods. Thymocytes and 3T3 fibroblasts attached to microcarrier beads were loaded with BCECF acetoxymethyl ester (BCECF-AME)²³ by the methods described in Fig. 1 legend for ^3H -quin 2-AME except that the media were supplemented with 2.5 mM NaHCO_3 and the incubation was with $1 \mu\text{M}$ BCECF-AME for 20 min. For BCECF-AME the spectral shift on hydrolysis is less pronounced (excitation maximum wavelength 480–505 nm; emission wavelength, 530 nm) and hydrolysis of this indicator was normally confirmed by obtaining the alkaline pH_i transient induced by the addition of 10 mM NH_4Cl to the medium¹⁴. The pH_i indicator BCECF was calibrated by permeabilizing the cells with 0.002% Triton X-100 and measuring fluorescence intensity as a function of the pH of the medium, titrated with HCl or Tris. Alternatively, the beads were allowed to sediment and the medium replaced with depolarizing medium (medium A but with 150 mM KCl substituted for NaCl and pre-warmed to 37°C) containing $2 \mu\text{M}$ of the K^+/H^+ ionophore nigericin. This approximately equilibrated the medium pH and pH_i (7.01), enabling the fluorescence signal to be calibrated as above.



below) or binding and endocytosis of EGF. It is therefore likely that the Ca^{2+} for the transient signal in response to EGF is from the external medium. In contrast, reducing $[\text{Ca}]_o$ to $<1 \mu\text{M}$ had only small effects on the Ca^{2+} signal in response to any of the other ligands (shown for VP in Fig. 1c). Most of the Ca^{2+} for these transient signals is therefore derived from intracellular Ca^{2+} pools. At higher EGTA concentrations that caused a net loss of Ca^{2+} from the cells and decreased $[\text{Ca}]_i$ to $<30 \text{ nM}$ after 1 h, the Ca^{2+} transient responses to VP and $\text{PGF}_{2\alpha}$ were reduced substantially.

Taken together, the data for quiescent thymocytes and fibroblasts show that both types of cell generate a transient Ca^{2+} signal in response to mitogenic concentrations of Con A, and that similar Ca^{2+} signals are generated by some of the receptor-specific mitogens which act on fibroblasts. The transient Ca^{2+} signals in both types of cell are independent of the extracellular Na^+ and K^+ concentrations in isotonic medium. The signals differ, however, in the extent to which they depend on $[\text{Ca}]_o$; the EGF signal is dependent entirely on $[\text{Ca}]_o$ whereas for the other mitogens, most of the Ca^{2+} is derived from the intracellular pool, as shown previously in fertilized sea urchin eggs⁹. Mitogenic concentrations of the Ca^{2+} ionophore A23187 ($20\text{--}40 \text{ nM}$)¹⁴ produced large Ca^{2+} signals in both types of cell which were maintained for at least 30 min and were dependent on $[\text{Ca}]_o$.

Although the various mechanisms by which the Ca^{2+} signals are generated in thymocytes, fibroblasts and eggs remain to be established, there are clearly striking similarities between the signals in the different types of cell. This prompted the question of whether a pH_i response occurs in thymocytes that have been allowed to become quiescent, corresponding to the pH_i increases reported for fibroblasts^{27,28}. It was also of interest to compare the time courses of the two ionic signals in the same experimental conditions. When quiescent thymocytes loaded with the pH_i indicators quene 1 (ref. 29) or 2',7'-bis(carboxyethyl)-5,6-carboxy fluorescein (BCECF)²³ were treated with Con A, there

was a delay of 1–2 min before any detectable increase in pH_i occurred (Fig. 2), and comparison with the time course of the Ca^{2+} signal shows that the rise in $[\text{Ca}]_i$ precedes the pH_i response. The pH_i responses to A23187 (which causes a rapid increase in $[\text{Ca}]_i$) and to TPA (which has little effect on $[\text{Ca}]_i$ in quiescent cells) are shown in Fig. 2b,c. Previous data using quene 1 or BCECF in freshly isolated thymocytes have shown no pH_i responses to Con A^{23,29}. We re-examined the response in freshly isolated cells after finding a substantial pH_i response in quiescent cells, and have detected marginal responses to Con A in some cell preparations and larger responses to TPA. This variability in the small pH_i signal in freshly isolated cells can probably be attributed to variations in the proportion of active cells in the population.

Figure 2d–f shows the pH_i changes in response to mitogens in samples of quiescent 3T3 cells loaded with BCECF. The stable fluorescence signal from the cells before stimulation indicates an intracellular pH of 7.01 ± 0.03 (s.e.m.; $n = 30$), in close agreement with the value obtained with 5,5-dimethyl oxazolidine-2-4 dione (DMO) measurement²⁷. Following addition of Con A, EGF, VP, $\text{PGF}_{2\alpha}$, A23187 or TPA, a rise in pH_i was detectable only after a few minutes and a stable pH_i of 7.14 ± 0.03 is reached after 10–15 min. Insulin, when added either alone or following the EGF-induced pH_i increase, induced a small but consistent increase of $\sim 0.05 \text{ pH}$ units. 8-Bromo-cyclic AMP, which is mitogenic for 3T3 fibroblasts, had no detectable effect on pH_i over 15 min. Some combinations of mitogens which generate a pH_i response separately gave approximately additive responses when added sequentially (Figs 2e,f, 3c).

The pH_i increase occurring with EGF is very similar to that described recently by Moolenaar *et al.*²⁸ in early-passage human foreskin fibroblasts using BCECF at 30°C and to the response to platelet-derived growth factor (PDGF) in NR-6 cells³⁰. The pH_i responses reported here for 3T3 fibroblasts also provide direct confirmation of those detected by DMO partition

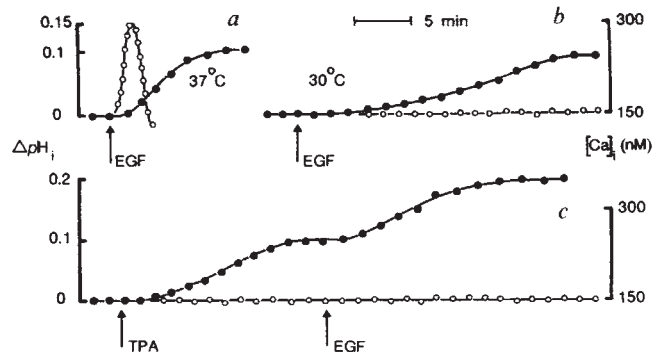


Fig. 3 Effects of temperature and TPA on $[Ca]_i$ and pH_i responses to EGF in Swiss 3T3 fibroblasts. pH_i (●) and $[Ca]_i$ (○) were measured as described in Figs 1 and 2 legends. *a*, Addition of EGF (12 ng ml^{-1}) at 37°C ; *b*, at 30°C ; *c*, TPA (10 nM) and EGF (12 ng ml^{-1}) added at 37°C as indicated.

measurement after 1 h with either VP or PDGF at 37°C ³¹. Note that much higher concentrations of A23187 ($>1 \mu\text{M}$) which inhibit mitogenic stimulation of fibroblasts¹⁴ cause pH_i to decrease, in agreement with the data of Moolenaar *et al.*²⁸. It therefore seems that the magnitude and time course of the pH_i response in different types of fibroblast is very similar.

In both thymocytes and fibroblasts, the pH_i response was independent of $[Ca]_o$ although in both types of cell Na^+ was required in the extracellular medium (Fig. 2c,e), consistent with previous reports for 3T3 and human foreskin fibroblasts^{27,28}. The response was maximal at $\sim 5 \text{ mM}$ and detectable at $>1 \text{ mM}$ Na^+ . We conclude that the pH response in thymocytes is probably generated by a Na^+/H^+ antiporter, as in fibroblasts^{27,31-33}.

These experiments demonstrate that agents which cause a Ca^{2+} signal in either cell type (Con A and A23187) also generate a pH_i signal, and the same relationship holds for the cell-specific mitogens which cause a Ca^{2+} signal in fibroblasts (EGF, VP and $\text{PGF}_{2\alpha}$). The Ca^{2+} signal precedes the pH_i signal, most clearly in the response of fibroblasts to EGF, VP, $\text{PGF}_{2\alpha}$ and Con A (see Fig. 2d), and detailed comparison of the time course of the two responses in the same cell preparations indicates that for all mitogens the rise in $[Ca]_i$ can be detected before the rise in pH_i .

The observation that a pH_i response invariably follows a Ca^{2+} signal suggested that the pH_i increase might be a direct consequence of the Ca^{2+} signal. The two ionic signals do appear to be coupled when they are generated by A23187, as both the

$[Ca]_i$ and pH_i responses are blocked when $[Ca]_o$ is reduced to $<1 \mu\text{M}$ (Fig. 2f). For all of the other ligands which generate both ionic signals in thymocytes or fibroblasts, however, the Ca^{2+} signal can be substantially diminished by prolonged incubation in low $[Ca]_o$, without significant effect on the pH_i response. In fibroblasts, the two signals in response to EGF can be uncoupled either by reducing the temperature to 30°C (Fig. 3a,b) or by lowering $[Ca]_o$ to $<1 \mu\text{M}$, which abolishes the Ca^{2+} signal (Fig. 1e) but not the pH_i response. In contrast, both $[Ca]_i$ and pH_i responses to VP and $\text{PGF}_{2\alpha}$ are retained at 30°C . Further evidence that the ionic signals are not directly coupled (other than in response to A23187) is provided by the effects of combinations of mitogens. TPA (10 nM) blocks the Ca^{2+} signal in response to EGF in fibroblasts, but the sequential addition of TPA and EGF gives approximately additive pH_i signals (Fig. 3c). Also, TPA, when added alone, causes the pH_i increase in both cell types without a Ca^{2+} signal, suggesting that activation of protein kinase C³⁴ generates the pH_i increase without a prior Ca^{2+} response, assuming that all actions of TPA at mitogenic concentrations ($\sim 10 \text{ nM}$) can be attributed to the activation of protein kinase C.

The implication that the pH_i and Ca^{2+} signals can be generated independently in response to receptor-mitogen interaction is consistent with the suggestion that both $[Ca]_i$ and pH_i signals can be generated from phosphatidylinositol 4,5-bisphosphate ($\text{PtdIns}(4,5)\text{P}_2$) breakdown to inositol 1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$) and diacylglycerol^{35,36}. The $\text{Ins}(1,4,5)\text{P}_3$ can release intracellular Ca^{2+} (ref. 36) and the diacylglycerol may activate protein kinase C³⁴ to cause the pH_i increase. Independent modulation of the two products, $\text{Ins}(1,4,5)\text{P}_3$ and diacylglycerol (or of the responses to them), would allow the ionic signals to be generated independently. We note, however, that mechanisms dependent on $\text{PtdIns}(4,5)\text{P}_2$ breakdown are unlikely to account for the ionic responses to all of the ligands: for example, EGF generates both ionic signals in 3T3 fibroblasts without causing detectable $\text{PtdIns}(4,5)\text{P}_2$ breakdown (ref. 37). Furthermore, EGF causes an additional increase in pH_i after the maximal pH_i response has been elicited by TPA, which strongly suggests that the pH_i response to EGF is not caused by activation of protein kinase C. A systematic analysis of the relationship between the ionic signals, polyphosphoinositide metabolism and mitogenic stimulation will be described elsewhere. The available evidence suggests, however, that the ionic components of the mitogenic pathways in eukaryotic cells are highly conserved.

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Structural organization of the rat *thy-1* gene

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Thy-1 is a differentiation marker expressed predominantly on thymocytes, T cells and brain tissue¹. Its presence on murine peripheral T cells but not B cells has long been used to distinguish between these two populations of lymphocytes². Although analogues of Thy-1 have been described in several mammalian species³⁻⁶, its tissue distribution in different species varies widely^{7,8}, precluding its use as T-cell-specific marker. The Thy-1 molecule is a cell-surface glycoprotein of relative molecular mass 18,000, one-third of which represents carbohydrate⁹; the protein moieties of the rat and murine Thy-1 molecules¹⁰ have been sequenced and found to consist of 111 and 112 amino acids, respectively. An unusual aspect of Thy-1 is the apparent absence of a hydrophobic segment comparable to that observed in other membrane glycoproteins which would allow integration of Thy-1 within the membrane lipid bilayer. This has prompted speculation that Thy-1 is anchored to the cell surface by some other hydrophobic component such as glycolipid. Here we report the structure of *thy-1* complementary DNA and genomic clones and describe the exon-intron organization of the gene. More importantly, our data indicate that Thy-1 is initially synthesized as a molecule of 142 amino acids, 31 amino acids longer at the carboxyl end than the Thy-1 molecule isolated and characterized by Campbell *et al.*¹¹. An extremely hydrophobic region of 20 amino acids lies within this 31-amino acid stretch and may represent the transmembrane segment responsible for anchoring Thy-1 to the cell membrane.

We have previously isolated a rat *thy-1* cDNA clone, pT64, which terminates prematurely at amino acid 103, preventing us from defining the carboxyl end of the molecule¹². Screening of a second rat thymocyte cDNA library using pT64 as a probe yielded an additional *thy-1* cDNA clone, pT86. The DNA sequence of the insert (Fig. 1) encodes the entire 111 amino acids of the Thy-1 molecule previously obtained by conventional protein sequencing methods. Surprisingly, however, the reading frame encoding this sequence continues for an additional 31 amino acids before reaching a termination codon (TGA). These 31 amino acids contain an extremely hydrophobic stretch of 20 amino acids (note the six consecutive leucine residues), which strongly resembles segments found in other membrane proteins. This unexpected observation prompted us to determine whether these extra 31 amino acids are also present in the normal *thy-1* gene.

A rat genomic library prepared in λ Charon 30 was screened with the *thy-1* cDNA clone, and of several positive clones obtained, one was selected for further structural analysis. Its DNA sequence was determined starting ~100 nucleotides 5' to the initiation codon of the gene. By comparing the genomic sequence with the sequences of the two cDNA clones and performing 3' S₁ nuclease mapping (data not shown), we deduced the intron-exon organization of the rat *thy-1* gene.

The coding sequence is distributed among three exons (Fig. 2) the first one shown (actually the second exon of the gene, because an intron is present in the 5'-untranslated part of the gene) encodes part of the 5'-untranslated region and the first 12 amino acids of the signal peptide. This is followed by an intron of 667 nucleotides, then another exon encoding the remainder of the signal peptide and amino acids 1-105 of the mature Thy-1 protein. Beyond this, there is then an additional intron of 402 nucleotides and an additional exon encoding amino acids 106-

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      1      10      20      30      40      50      60      70      80      90
A  Gln Arg Val Ile Ser Leu Thr Ala Cys Leu Val Asn Gln Asn Leu
   CAG AGG GTG ATC AGC CTG ACA GCC TGC CTG GTG AAC CAG AAC CTT

      20      30      40      50      60      70      80      90
Arg Leu Asp Cys Arg His Glu Asn Asn Thr Asn Leu Pro Ile Gln His
CGA CTG GAC TGC CGT CAT GAG AAT AAC ACC AAC TTG CCC ATC CAG CAT

      40      50      60      70      80      90
Glu Phe Ser Leu Thr Arg Glu Lys Lys His Val Leu Ser Gly Thr
GAG TTC AGC CTG ACC CGA GAG AAG AAG AAC CAC CTG GTG TCA GGC ACC

      50      60      70      80      90
Leu Gly Val Pro Glu His Thr Tyr Arg Ser Arg Val Asn Leu Phe Ser
CTG GGG GTT CCC GAG CAC ACT TAC CGC TCC CGC GTG AAC CTT TTC AGT

      70      80      90
Asp Arg Phe Ile Lys Val Leu Thr Leu Ala Asn Phe Thr Thr Lys Asp
GAC CGC TTT ATC AAG GTC CTT ACT CTA GCC AAC TTC ACC ACC AAG GAT
      Ava I

      80      90
Glu Gly Asp Tyr Met Cys Glu Leu Arg Val Ser Gly Gln Asn Pro Thr
GAG GGC GAC TAC ATG TGT GAA CTT CGA GTG TCG GGC CAG AAT CCC ACA
      Ava I

      100      110
Ser Ser Asn Lys Thr Ile Asn Val Ile Arg Asp Lys Leu Val Lys Cys
AGC TCC AAT AAA ACT ATC AAT GTG ATG AGA GAC AAG CTG GTC AAG TGT
Alu I      Sau 3A

      120      130      140
Gly Gly Ile Ser Leu Leu Val Gln Asn Thr Ser Trp Leu Leu Leu Leu
GGT GGC ATA AGC CTG CTG GTT CAA AAC ACT TCC TGG CTG CTG CTG
      CTG

      130      140
Leu Leu Ser Leu Ser Phe Leu Gln Ala Thr Asp Phe Ile Ser Leu *
CTG CTT TCC CTC TCC TTC CTC CAA GCC ACG GAC TTC ATT TGT CTG TGA
CTGGTTGGGC CC AAGGAGAA ACAGGAAACC TCAAGGTCGTG CTGAGAGGT CTTCCTTC
      Sau 961

TC CCGGTGAGT GACTGCTTCC CCAAGACCTT CAAATATCTC AAAACGGGG GAGAA
      Hinf I

ATGG

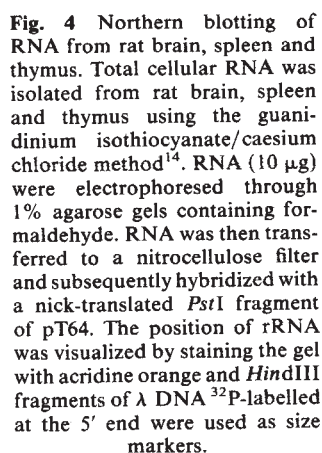
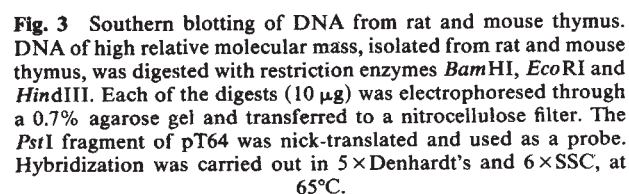
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Fig. 1 Nucleotide sequence of the cDNA insert of pT86 and the predicted amino acid sequence of the complete rat Thy-1 antigen. The cDNA library was constructed using mRNA from W/Fu rat thymocytes as described previously¹². One hybridization-positive clone (pT86) was isolated from ~10,000 colonies using the entire *Pst*I insert of the first *thy-1* clone, pT64, as a probe. A restriction map was constructed based on the size of DNA fragments obtained after restriction endonuclease digestion. The entire DNA of pT86 was cleaved at selected sites with restriction endonucleases (BRL) and fragments corresponding to the insert were purified by acrylamide gel electrophoresis and electroelution. Purified fragments were treated with calf intestinal alkaline phosphatase (Boehringer), ³²P-labelled at both 5' ends with T4 polynucleotide kinase plus [³²P]ATP as described previously¹² and cleaved secondarily to generate subfragments with only one labelled end. The restriction enzyme sites shown and both *Pst*I sites are those that were labelled at the 5' end. The subfragments were separated by acrylamide gel electrophoresis, electroeluted and subjected to partial chemical degradation sequence analysis as described by Maxam and Gilbert¹³. Both strands of the insert cDNA were sequenced. The hydrophobic 20-amino acid segment is underlined and the termination codon is indicated by an asterisk.

142 plus the termination codon, TGA. Two polyadenylation signals, AAUAAA, are located 569 and 1,055 nucleotides, respectively, downstream from the termination codon, although only the latter one is actually used for polyadenylation. Thus, the sequence of the rat *thy-1* gene is perfectly consistent with the cDNA sequence and strongly suggests that rat Thy-1 is synthesized initially as a polypeptide of 142 amino acids rather than 111 amino acids. We have recently isolated and sequenced the mouse and human *thy-1* genes and find that both also contain an additional 31 amino acids at the carboxyl end (T.S. *et al.*, manuscripts in preparation).

To eliminate the possibility of a second *thy-1* gene encoding a molecule of 111 amino acids or differential processing of a *thy-1* nuclear transcript which would give rise to a second messenger RNA encoding a Thy-1 molecule of 111 acids, Southern and Northern blots were performed. The Southern blots (Fig. 3) indicate the presence of a single *thy-1* gene in both the rat and mouse genome. Similarly, Northern blots (Fig. 4) demonstrate the existence of a single mRNA species of ~1.85 kilobases (kb) in brain and thymus tissue. This mRNA hybridizes to a nick-translated DNA fragment corresponding to amino acids 106-142 of the Thy-1 molecule (data not shown) and thus includes the extra 31 amino acids observed in the cDNA and genomic clones. There is no evidence for a second smaller

Fig. 2 Nucleotide sequence of the rat *thy-1* gene. A genomic library was prepared in the λ vector Charon 30 using a partial *Mbo*I digest of rat thymus DNA. The isolated gene was sequenced according to Maxam and Gilbert¹³. The underlined portions represent the exons deduced from the cDNA sequences and the 3' S₁ nuclease mapping. The protein sequence is numbered from -19 to -1 (signal peptide) and 1-142. The two polyadenylation signals at nucleotide positions 2,154 and 2,641 are indicated by double lines. Asterisks indicate the termination codon.



mRNA encoding a shorter Thy-1 molecule. S₁ nuclease mapping (data not shown) indicates that the large size of the mRNA is the result of a large 3' untranslated region (1,062 nucleotides) extending from the end of the coding region to the second polyadenylation signal.

There are two possible reasons for the discrepancy between the cDNA and genomic data and the protein sequence data of Williams and Gagnon¹⁰. First although the purified Thy-1 molecule is indeed 142 amino acids long, the hydrophobic peptide containing the extra 31 amino acids may have been lost during the preparation and purification of the peptide fragments used for sequencing. Alternatively, there may be a processing step in which the newly synthesized Thy-1 molecules are cleaved to yield mature molecules of a different size. Pulse-chase experiments to explore the latter possibility further failed to reveal any post-translational proteolytic processing. Furthermore, when ³H-tryptophan is used for incorporation, the mature Thy-1 molecule is visible even after an extensive (30 min) chase (T.S. *et al.*, manuscript in preparation). Thus, the mature Thy-1 molecule extends beyond the 111 amino acids proposed by Campbell *et al.*¹¹ and up to at least amino acid 123, the position of the sole tryptophan residue. These data suggest strongly that Thy-1 is anchored to the cell surface by a conventional hydrophobic transmembrane segment. It is intriguing, however, that the intracytoplasmic segment described for other membrane glycoproteins is absent and the functional significance of this remains unknown.

This work was supported by NIH grant CA 38404.

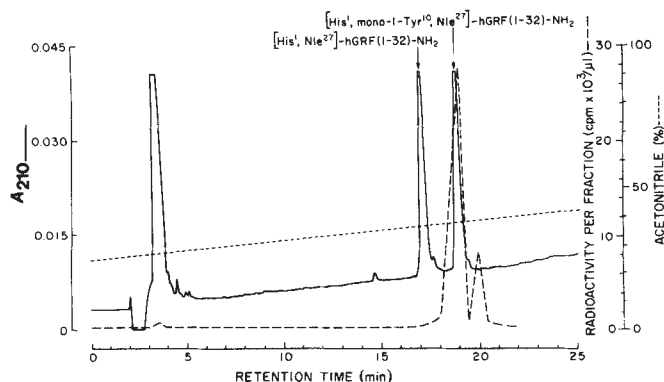


Fig. 1 Reverse-phase HPLC elution profile of 5 µg [His¹, mono-I-Tyr¹⁰, Nle²⁷]-hGRF(1-32)-NH₂ and 5 µg [His¹, Nle²⁷]-hGRF(1-32)-NH₂, detected by absorbance at 210 nm. [His¹, Nle²⁷]-hGRF(1-32)-NH₂ was radioiodinated and purified, then analysed in the same run. The separation was carried out on a C₁₈ column (Vydac, 0.46 × 25 cm, pore size 300 Å) in a linear gradient of acetonitrile in H₂O and 0.1% trifluoroacetic acid (24–42% CH₃CN in 25 min, flow rate 1.5 ml min⁻¹). Fractions of the eluate were collected every 0.5 min and radioactivity per fraction determined. [His¹, Nle²⁷]-hGRF(1-32)-NH₂, as well as all GRF analogues and other peptides, was synthesized by solid phase methods as described elsewhere¹ and iodinated using chloramine-T (ref. 18). Final HPLC of the iodinated product was carried out after an initial purification of the iodination product on a disposable C₁₈ cartridge (BondElut, Analytichem). Two radioactive peaks were eluted well separated from the unlabelled analogue. The second smaller peak, as it increased with prolonged iodination times, probably represented the diiodinated analogue. The first peak, which normally eluted in one tube, was diluted with H₂O to ~10⁵ c.p.m. µl⁻¹ and stored at -20 °C after addition of bovine serum albumin (Pentex) at a final concentration of 1% (w/v). After its iodination, the radioligand was used for up to 3 days, after which time re-purification on C₁₈ columns and HPLC was carried out.

From our structure-function studies and knowing the sequence of rGRF, which has a histidine in the N-terminal position, we anticipated that the analogue [His¹, Nle²⁷]-hGRF(1-32)-NH₂ would be of high binding potency. We chose this analogue in order to avoid iodination of the amino-terminal tyrosine (in hGRF) and possible oxidation of the methionine in position 27 of the native peptide. The radioligand, prepared as described in Fig. 1 legend, is assumed to be [His¹, mono-¹²⁵I-Tyr¹⁰, Nle²⁷]-hGRF(1-32)-NH₂ (specific activity ~570 µCi per µg peptide) because, on resolute HPLC systems, it co-elutes with the non-radioactive [His¹, mono-I-Tyr¹⁰, Nle²⁷]-hGRF(1-32)-NH₂ synthesized *de novo*.

We studied GRF binding to fresh rat anterior pituitary cells as a function of time and temperature (Fig. 2). At 37 °C, maximal specific binding was reached within 20 min, followed by a rapid decline. At 23 °C, specific binding reached equilibrium after 30 min and remained stable for at least 2 h. At 4 °C, specific binding, obtained by 60 min, was only half that at 23 °C. Specific binding was fully reversible by adding 10⁻⁷ M unlabelled ligand at 30 min. All subsequent experiments were based on a 30-min incubation at 23 °C.

To assess tracer integrity, the radioligand was incubated in the presence of cells under standard assay conditions, and ligand stability was assessed with HPLC after acid extraction. Of the ligand recovered from the incubation supernatant, >80% was intact, whereas 98% of the ligand extracted from cell pellets was identical to ligand exposed to buffer only.

Availability of the non-radioactive mono-iodinated synthesized ligand enabled us to determine affinity in competition studies. A dissociation constant (*K*_D) of 41 × 10⁻¹² M (11–160 × 10⁻¹² M) and an estimated receptor concentration of 11 fmol per pituitary equivalent (4–29 fmol) were derived from analysis of four competition experiments using the computerized, non-

Binding sites for growth hormone releasing factor on rat anterior pituitary cells

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Growth hormone releasing factors (GRFs) have been isolated from human pancreatic tumours (hGRF)^{1–3} and rat hypothalamus (rhGRF)⁴. The response to GRF at the pituitary level can be modulated by other factors, including glucocorticoids⁵, thyroid hormones⁵, somatostatin and other neuropeptides^{5,6} and somatomedins^{7,8}. Glucocorticoids enhance GRF-induced growth hormone (GH) secretion in primary cultures of rat anterior pituitary cells^{5,9}, and the synthetic glucocorticoid dexamethasone has recently been shown to increase the amounts of GH released in freely moving rats in response to submaximal doses of intravenous GRF¹⁰. To investigate whether somatotroph sensitivity to GRF is modulated at its receptor level, we have developed a radioreceptor assay using an iodinated analogue of hGRF as radioligand. We report here that the relative binding affinities of rGRF, hGRF and the two analogues are correlated with their *in vitro* biological potencies. Further, the number of GRF binding sites is drastically decreased in cells deprived of glucocorticoids either *in vivo* or *in vitro*.

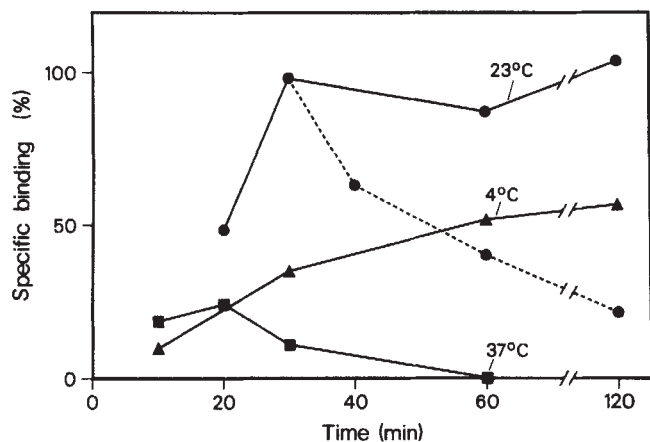


Fig. 2 Time course of specific $[\text{His}^1, \text{mono}^{125}\text{I-Tyr}^{10}, \text{Nle}^{27}]$ -hGRF(1-32)-NH₂ binding to freshly dispersed rat anterior pituitary cells (●, ▲, ■) at temperatures indicated. ●—●, Reversibility of specific binding after addition of 10^{-7} M $[\text{His}^1, \text{Nle}^{27}]$ -hGRF(1-32)-NH₂ at 30 min. Rat anterior pituitary cells from male Sprague-Dawley rats (200–220 g) were dispersed as described previously²¹. Approximately 1.5×10^6 viable cells were obtained per animal. After dispersion, cells were rinsed twice with, and finally suspended in, standard buffer (50 mM Tris-HCl pH 7.4, 2 mM EDTA, 5 mM MgCl₂, 0.24 M sucrose) and used for binding experiments not longer than 3 h after dispersion. For binding studies, 1.0 – 1.5×10^6 suspended cells and about 10 fmol radioligand were incubated in a final volume of 0.5 ml standard buffer (duplicates, borosilicate glass tubes, 12 × 75 mm). The reaction was terminated by immersing the incubation tube in ice water and transferring 0.45 ml to an ice-chilled polyethylene microfuge tube. After 2 min centrifugation (Beckmann microfuge) at 4 °C, the supernatants were aspirated, the tips of the tubes containing cell pellets cut off and counted in a γ counter. Generally, 8–12% of the total radioactivity was bound, the background (no tissue) being <1%. Of the total binding, 50–70% was displaceable in the presence of 10^{-7} M $[\text{His}^1, \text{Nle}^{27}]$ -hGRF(1-32)-NH₂ (= specific binding). There was no specific binding to test tubes or microfuge tubes.

linear regression analysis, LIGAND¹¹. Figure 3 shows a representative competition curve. To demonstrate pharmacological specificity of the GRF binding site, we examined several GRF analogues and unrelated peptides for their ability to compete with the radioligand. Binding affinities of different GRFs and homologous peptides correlated with their GH releasing potency *in vitro* (Table 1). The binding site had higher affinity for rGRF than for hGRF(1-40). C-terminally-amidated shortened analogues displayed highest binding affinity and *in vitro* potency, whereas deletion of the amino-terminal residue resulted in an almost complete loss of biological and binding activity. The analogue $[\text{His}^1, \text{mono-I-Tyr}^{10}, \text{Nle}^{27}]$ -hGRF(1-32)-NH₂ retained full *in vitro* potency. Homologous peptides from the secretin-glucagon family, PHI²² and vasoactive intestinal polypeptide (VIP), did not displace the ligand at concentrations up to 10^{-7} M. In agreement with biological studies, this may indicate a different specificity of pituitary binding sites compared with pancreatic receptors that bind VIP and rGRF with high affinity¹². Regional specificity of hormone receptors may have developed in parallel with evolving families of homologous peptides.

The effect of glucocorticoid deprivation on GRF binding was examined in freshly dispersed cells from adrenalectomized and sham-operated rats [Fig. 3]. One week after adrenalectomy, pituitary GRF binding capacity was decreased to 26% that of control cells ($26 \pm 5\%$ s.e.; $P < 0.01$, $n = 6$). Because affinities in both groups did not differ significantly, the lower binding capacity in cells after adrenalectomy must have been caused by a decreased number of binding sites. From *in vitro* observations³, we anticipated that glucocorticoids might act directly at the pituitary level to modulate GRF receptor numbers and have examined this possibility by investigating the effects of dexamethasone on cultured anterior pituitary cells. The maximal specific binding to cells maintained in glucocorticoid-free

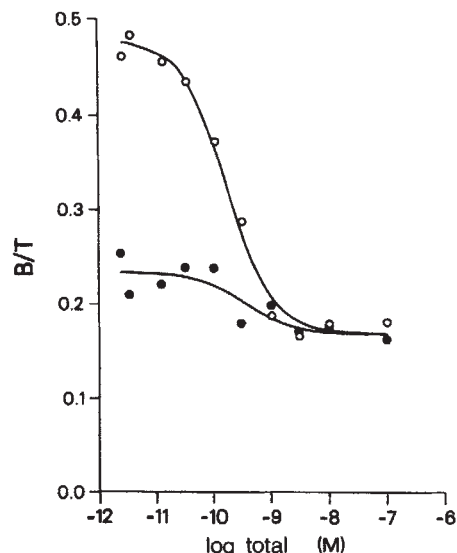


Fig. 3 Binding competition study comparing cells from sham-operated animals (○) and cells from rats 1 week after bilateral adrenalectomy (●). Binding experiments were carried out in standard conditions, incubating 1.3×10^6 freshly dispersed cells in the presence of a constant amount of radioligand and increasing concentrations of $[\text{His}^1, \text{mono-I-Tyr}^{10}, \text{Nle}^{27}]$ -hGRF(1-32)-NH₂. The data shown were obtained from one population of rats and in the same experiment performed in duplicate, the curves were fitted and analysed using the program LIGAND^{11,20}. The curve for sham-operated animals is representative of competition curves obtained from normal fresh cells. Estimated receptor concentrations in the experiment shown were: controls, 17 fmol per 10^6 cells, adrenalectomized, 3.8 fmol per 10^6 cells (22% of control). The calculated affinities were not significantly different: 73 pM (43–125 pM) in controls, 144 pM (28–720 pM) in adrenalectomized animals. B/T, bound to total analogue present.

medium for 24 h was found to be significantly less than in control cells cultured in the presence of 5 nM dexamethasone ($30 \pm 12\%$ compared with $100 \pm 20\%$, $P < 0.01$).

Thus, a radioreceptor assay for GRF has been established using an iodinated 'tailor-made' analogue as radioligand. Binding experiments were preferentially carried out using freshly dispersed rat anterior pituitary cells, because cultured cells show decreased specific GRF binding with time in culture, as described earlier for gonadotropin releasing hormone binding

Table 1 Relative biological potencies and binding affinities of different peptides

Peptide	Relative biological potency <i>in vitro</i>	Relative binding affinity
hpGRF(1-40) (standard)	1.0	1.0
rGRF	2.7(1.8–4.1)	5.8(2.5–13.4)
$[\text{His}^1, \text{Nle}^{27}]$ -hGRF(1-32)-NH ₂	3.2(1.8–5.7)	4.8(2.1–11.4)
$[\text{His}^1, \text{mono-I-Tyr}^{10}, \text{Nle}^{27}]$ -hGRF(1-32)-NH ₂	4.9(3.2–7.3)	9.7(2.9–32)
desTyr ¹ -hGRF(2-32)-NH ₂	<0.001	<0.01
VIP	<0.001	<0.01
PHI	<0.001	<0.01

Biological potencies were determined by comparison of the GH response of rat anterior pituitary cell primary cultures¹⁸ to increasing concentrations of hGRF(1-40) and to the respective analogue. Relative biological potency and 95% confidence limits were calculated with the program BIOPROG¹⁹. Binding affinities with 95% confidence limits were determined using the program LIGAND¹¹ for analysis of competition studies. (Confidence limits ($P < 0.05$) for data from the LIGAND program were calculated according to ref. 20.) At least three experiments per analogue were carried out in duplicate.

sites on pituitary cells¹³. Binding affinities of several GRF-related peptides correlate with their *in vitro* potencies.

Glucocorticoids seem to be essential for the somatotroph to maintain a normal number of GRF binding sites. Previously, glucocorticoids have been reported to stimulate GH production and GH gene transcription in GH-producing pituitary tumour cells¹⁴⁻¹⁶ and to increase the sensitivity of normal rat pituitary cells to GRF *in vitro*^{5,9} and *in vivo*¹⁰. As glucocorticoids also decrease the sensitivity to somatostatin in normal pituitary cells⁵, decrease somatostatin receptors on cultured GH-secreting pituitary tumour cells¹⁷ and increase GRF binding sites (this study), glucocorticoids may act in a coordinated fashion at the pituitary level to increase GH secretion.

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Cloning and sequence analysis of a cDNA for rat transforming growth factor- α

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Transforming growth factors (TGFs) are mitogenic polypeptides produced most conspicuously by transformed cells and conferring on normal cells several phenotypic alterations associated with transformation^{1,2}. TGFs comprise two distinct sets of molecules: TGF- α s are structurally similar to epidermal growth factor (EGF), binding to and inducing the tyrosine phosphorylation of the EGF receptor in a manner indistinguishable from that of EGF³. In addition, the 50-amino acid rat TGF- α ⁴ has 33 and 44% homologies with mouse⁵ and human⁶ EGFs, respectively, and shares with EGFs a conserved pattern of three disulphide bridges⁷. Thus, it has been proposed that TGF- α s belong to a family of EGF-like polypeptides⁷. TGF- β s, on the other hand, display no measurable binding to EGF receptors, but potentiate the growth-

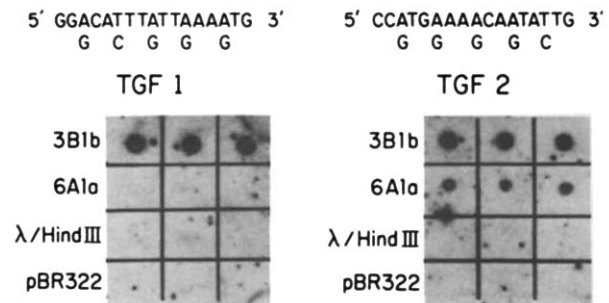


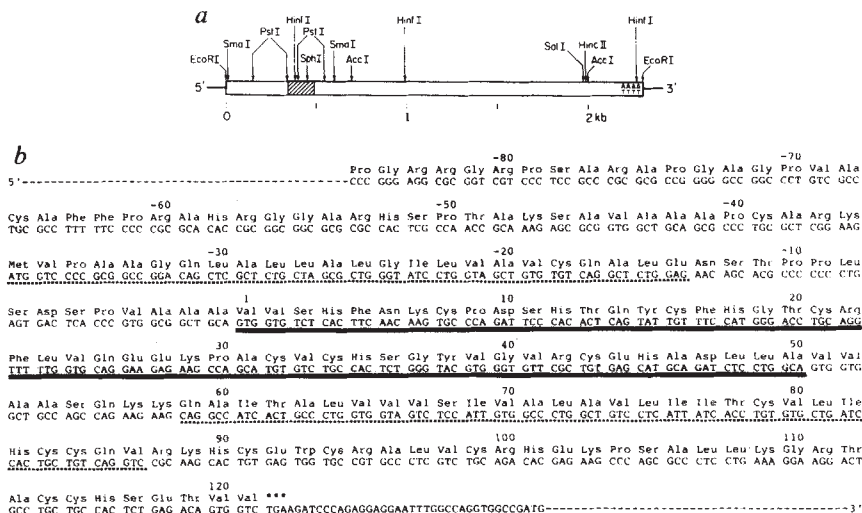
Fig. 1 Identification of a rat TGF- α cDNA clone. Poly(A)⁺ RNA was isolated from FeSV-transformed Fisher rat embryo cells and used to synthesize cDNA. First-strand cDNA synthesis was performed using oligo(dT) and avian myeloblastosis virus reverse transcriptase (Molecular Genetic Resources, Inc.) and second-strand using *Escherichia coli* RNase H, DNA polymerase I and ligase (NAD⁺)¹⁸. The double-stranded cDNA was digested with S₁ nuclease, end-filled with the large fragment of DNA polymerase I, treated with *Eco*RI methylase and ligated to *Eco*RI synthetic linkers. Following digestion with *Eco*RI, cDNAs >500 bp were selected by chromatography on Bio-Gel A50 and precipitated with ethanol in the presence of *Eco*RI-digested λ gt10¹² DNA. Resuspended DNAs were ligated overnight at 15 °C, packaged *in vitro* and used to infect *E. coli* strain C600 rk⁺ Hfl. Infected cells were plated at a density of 20,000 plaque-forming units per 150-mm dish and nitrocellulose plaque-lifts were prepared from each plate in duplicate. Filters were probed with a pool of two synthetic oligonucleotides (TGF-1, TGF-2), each of which consisted of a mixture of 32 independent sequences. The oligonucleotides were labelled using [α -³²P]ATP (NEN) and T4 polynucleotide kinase. Hybridization was carried out at 37 °C in 5 $\times$ SSC, 5 $\times$ Denhardt's reagent, 0.05% sodium pyrophosphate and 100 μ g ml⁻¹ transfer RNA at 37 °C with 10⁷ c.p.m. ml⁻¹; washes were at 37 °C in 6 $\times$ SSC. Two positive clones (3B1b, 6A1a) were plaque-purified and DNA (10 ng) isolated from these clones was spotted (in triplicate) onto duplicate nitrocellulose filters with equal amounts of λ and pBR322 DNAs. The filters were probed with labelled TGF-1 (left) and TGF-2 (right), respectively. Hybridization was carried out as described above and washes were at 45 °C in 6 $\times$ SSC.

stimulating activities of TGF- α ⁸. Here we report the isolation of a complementary DNA clone encoding rat TGF- α . This cDNA hybridizes to a 4.5-kilobase (kb) messenger RNA that is 30 times larger than necessary to code for a 50-amino acid polypeptide and is present not only in retrovirus-transformed rat cells but also at lower levels in normal rat tissues. The nucleotide sequence of the cDNA predicts that TGF- α is synthesized as a larger product and that the larger form may exist as a transmembrane protein. However, unlike many polypeptide hormones (including EGF^{9,10}), cleavage of the 50-amino acid TGF- α from the larger form does not occur at paired basic residues, but rather between alanine and valine residues, suggesting the role of a novel protease.

Two mixtures of oligonucleotides, each 17 nucleotides long, were prepared as hybridization probes to identify cDNA clones containing TGF- α sequences. The first, TGF-1 (Fig. 1), encoded amino acids 4-9 of the rat TGF- α sequence⁷, whereas the second, TGF-2, corresponded to the region of amino acids 14-19. Because of codon ambiguity, each oligonucleotide mixture contained 32 different DNA sequences, only one of which had perfect homology with the TGF- α gene.

Double-stranded cDNA was synthesized using poly(A)⁺ RNA isolated from a clone (ST FeSV FRE Cl. 10; ref. 11) of feline sarcoma virus (FeSV)-transformed Fisher rat embryo cells (see Fig. 1), attached to synthetic *Eco*RI linkers and ligated to *Eco*RI-digested λ gt10 DNA¹². Screening of 150,000 recombinant plaques with a mixture of both 17-mers yielded two duplicating positive clones which were further purified by secondary and tertiary screening. DNA was isolated from these two clones (3B1b and 6A1a), adsorbed onto nitrocellulose and duplicate sets of these DNAs were probed separately with either TGF-1 or TGF-2. λ gt10 clone 3B1b hybridized strongly to both 17-mers, whereas 6A1a hybridized only to TGF-2 and did so

Fig. 2 *a*, Structural features of the rat TGF- α cDNA clone. A partial restriction map was determined for the insert (shown as the open bar) from clone prTGF $_{0.2}$; the 5' and 3' terminal *Eco*RI sites result from the addition of *Eco*RI linkers during the cloning process. The orientation of the clone is indicated, as is the presence of a 20-residue poly(dA: dT) region at the 3' end corresponding to the polyadenylation site of the mRNA. The cross-hatched area indicates the location of the 50-amino acid TGF- α coding sequence. *b*, Nucleotide sequence of the TGF- α coding region of prTGF $_{0.2}$ was determined by dideoxynucleotide sequencing¹⁹. The amino acid sequence of rat TGF- α ⁷ is delineated by the heavy underlining, and extended regions of uncharged and hydrophobic residues are underlined with dots. Asterisks indicate the termination codon.



with a weaker affinity than 3B1b (Fig. 1). Thus, only 3B1b was analysed further.

The 2.3-kb *EcoRI* insert from 3B1b was subcloned into the plasmid pEMBL (ref. 13); this recombinant was termed prTGF_{0,2} (see Fig. 2 for a schematic representation). Southern analysis of the cDNA insert digested with various restriction enzymes revealed that both oligonucleotide probes hybridized to the region denoted by cross-hatching in Fig. 2a (data not shown). The nucleotide sequence of the entire prTGF_{0,2} insert was determined and the cross-hatched region of Fig. 2a was found to encode an open reading frame including a stretch of 50 amino acids identical in sequence to that published previously for rat TGF- α ⁷. The absence of both an encoded initiating methionine immediately preceding the amino-terminal valine of rat TGF- α and a termination codon following the carboxy-terminal alanine suggests that, as is the case for many polypeptide hormones, TGF- α is synthesized initially as a larger protein. Although the size of the initial translation product cannot be deduced unambiguously from an analysis of this partial cDNA clone, the 50-amino acid TGF- α sequence is preceded by an encoded methionine lying 38 amino acids upstream and is followed by 71 amino acids before a stop codon occurs. If this methionine corresponds to the initiator, the TGF- α translation product would comprise 159 amino acids. Consistent with this suggestion, antibodies raised against a synthetic peptide corresponding to the carboxy-terminus of TGF- α bind most conspicuously in immunoblots of proteins secreted by FeSV-transformed rat cells to a larger polypeptide of relative molecular mass (M_r) $\sim$ 20,000 (ref. 14). In addition, the cDNA sequence predicts that the methionine is followed immediately by a region of approximately 25 largely uncharged or hydrophobic residues characteristic of signal peptides.

A second extremely hydrophobic domain begins ~10 residues after the carboxy-terminus of the 50-amino acid TGF- α . This region, which consists almost exclusively of isoleucines, leucines and valines, is characteristic of transmembrane cores and is similar with respect to both its hydrophobicity and its position following the TGF- α sequence to a hydrophobic region carboxy-terminal to the EGF sequence within its precursor^{9,10}. The second hydrophobic domain is then followed by a non-hydrophobic, cysteine-rich sequence of ~40 residues. The location of this second hydrophobic region suggests that the initial TGF- α translation product serves as a transmembrane protein possessing biologically active polypeptides on both sides of the membrane. This larger form of TGF- α may interact with the EGF receptor within the same membrane and/or itself function as a membrane receptor.

Additional analysis of the prTGF_{0,2} sequence suggests several interesting features regarding the possible processing of the larger TGF- α . In contrast to the processing of most polypeptides, including EGF^{9,10}, the cleavage sites which surround the 50-

amino acid TGF- α are not bordered by either single or paired basic amino acids (for example, Lys, Arg). Rather, the cleavage sites which release the 50-amino acid TGF- α are embedded in alanine-valine-rich regions, with the actual cleavages occurring between alanine and valine residues at both the amino- and carboxy-termini. This type of proteolytic specificity is characteristic of elastases, which seem to cleave the carbonyl bond of amino acids possessing uncharged, non-aromatic side chains, like alanine and valine¹⁵. Other possible candidates include the metal-containing, neutral proteases (thermolysin-like) which cleave frequently at sites immediately preceding valine residues¹⁶, but have not yet been demonstrated in animal cells, as well as signal peptidases¹⁷ which cleave in regions of hydrophobic amino acids and recognize conformation rather than specific sequence. On the other hand, it is interesting that the second hydrophobic core is bordered at both ends by paired basic residues (Lys-Lys at positions +57, 58 and Arg-Lys at positions +88, 89) which, as described above, might serve as recognition sites for proteolytic cleavage; this in turn suggests that the carboxy-terminal, cysteine-rich polypeptide is released inside the cell as a biologically active molecule and that the activity of the original TGF- α translation product might be regulated by two entirely different types of proteases.

Consistent with the existence of a larger TGF- α translation product, we observed that its mRNA is 20-30-fold larger than

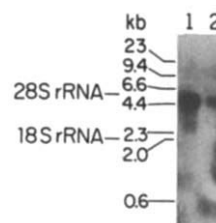


Fig. 3 Northern blot analysis of rat poly(A)⁺ RNAs probed with the TGF- α cDNA clone. RNA was isolated by homogenization of tissue or cells in 4 M guanidine thiocyanate and sedimentation through 5.7 M CsCl (refs 20, 21). Poly (A⁺) RNA was selected by chromatography on oligo(dT) cellulose and aliquots (5 μ g) electrophoresed through 1% agarose gels containing formaldehyde²². RNA was transferred to nitrocellulose and probed with the nick-translated *Eco*RI insert from prTGF_{0.2}. Hybridization was carried out at 45°C in 50% formamide, 1× Denhardt's reagent, 5× SSC, 25 mM NaH₂PO₄, 200 μ g ml⁻¹ herring sperm DNA and washes were 2× SSC, 0.1% SDS at 68°C. The markers (in kb) are *Hind*III-digested λ DNA and 18S and 28S ribosomal RNAs, which are ~2 and 5 kb in length, respectively. Poly(A)⁺ RNAs are from FeSV-transformed Fisher rat embryo cells (lane 1) and adult female rat brain (lane 2).

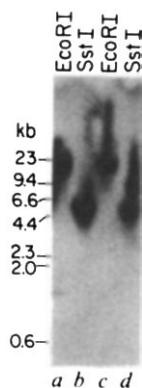


Fig. 4 Southern blot of rat genomic DNA probed with the TGF- α cDNA clone. DNA was isolated from adult female rat kidney (lanes *a*, *b*) and FeSV-transformed Fisher rat embryo cells (lanes *c*, *d*) and restricted with the indicated enzymes. Digested DNA (20 μ g) was electrophoresed on a 1% agarose gel, transferred to nitrocellulose by the method of Southern²³ and probed with the nick-translated *EcoRI* insert from pTGF_{0.2}. Hybridization and washes were carried out as described in Fig. 3 legend. The markers (in kb) are *HindIII*-digested λ DNA.

necessary to code for a 50-amino acid polypeptide. Northern blots of poly(A)⁺ RNA obtained from FeSV-transformed rat cells, when probed with the *EcoRI* insert from pTGF_{0.2}, yielded a single band of ~4.5 kb (Fig. 3). In addition, as sequence analysis of the 3' end of the pTGF_{0.2} insert revealed a 20-residue poly(A) tract, the cloned cDNA apparently corresponds to the second half of the TGF- α mRNA. The 50-amino acid TGF- α is encoded by a sequence located roughly 2.5 kb from the 5' end of the mRNA.

Northern blot analysis of poly(A)⁺ RNA from normal rat brain, when probed with the TGF- α clone, revealed a single band identical in size to that obtained from the FeSV-transformed cells (Fig. 3). The level of TGF- α -specific mRNA relative to total poly(A)⁺ RNA in brain was roughly 10–20-fold lower than that in FeSV-transformed cells (data not shown). In addition, we have observed TGF- α mRNA in rat kidney and liver in amounts similar to that in brain (data not shown). The presence of TGF- α -specific mRNA in tissues raises interesting questions regarding the role of this polypeptide in normal cells.

As described above, TGF- α is structurally homologous to EGF, and both bind, in an equivalent fashion, the EGF receptor. This similarity is extended further by a comparison of the cDNAs encoding EGF^{9,10} and TGF- α which indicates that both polypeptides are synthesized as larger proteins encoded by mRNAs of 4.5–5.0 kb. In addition, both growth factors share striking homology with a 140-base pair vaccinia virus protein of unknown function (J. Brown *et al.*, accompanying paper²⁴). As both growth factors appear, therefore, to belong to a family of EGF-like polypeptides, we used the TGF- α cDNA to screen Southern blots of rat genomic DNA to identify homologous genes in this family. In moderate stringency conditions, however, only one (*SstI*) or two (*EcoRI*); the second band of ~1.0 kb may not be visible) bands appeared with each restriction enzyme tested (Fig. 4), indicating that the TGF- α gene is probably unique in rats and is not highly homologous to genes encoding other related polypeptides. In addition, a comparison of the results obtained with DNAs isolated from kidney and FeSV-transformed cells indicated that elevated levels of TGF- α mRNA in the latter are not the result of gross amplification or rearrangement of the gene.

We thank Jane Ranchalis for providing FeSV-transformed Fisher rat embryo cells, Daniel Twardzik, Hans Marquardt, Peter Linsley, Debra Bryant, Hans Neurath and Kenneth Walsh for helpful discussions, and Lynda Taylor for assistance in preparing the manuscript.

Note added in proof: After this manuscript had been submitted, the cloning of human TGF- α was reported²⁵.

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Vaccinia virus encodes a polypeptide homologous to epidermal growth factor and transforming growth factor

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Epidermal growth factor (EGF) and transforming growth factor type I (TGF) are polypeptides of 53 and 50 amino acid residues, respectively^{1,2}. Both bind to EGF receptor, a 1,200-residue transmembranous glycoprotein³, leading to phosphorylation of the receptor, enhancement of its tyrosine-specific kinase activity and ultimately to stimulation of cell growth^{4,5}. We report here that a 140-residue polypeptide encoded by one of the early genes of vaccinia virus (VV)⁶ is related closely to EGF and TGF. The presence of putative signal and transmembranous sequences further suggests that the viral protein might be an integral membrane protein, but that, as in the case of EGF itself^{7,8}, the membrane-associated form may be the precursor of a soluble growth factor. Production of EGF-like growth factors by virally infected cells could account for the proliferative diseases associated with members of the poxvirus family such as Shope fibroma virus⁹, Yaba tumour virus¹⁰, and molluscum contagiosum virus (MCV)¹¹.

The Dayhoff protein sequence library of 2,676 sequences (Protein Identification Resource, Georgetown, Washington, DC) was searched for sequences related to rat TGF (rTGF). The three most closely related sequences were mouse EGF (mEGF)^{7,8} and human EGF (hEGF, urogastrone)¹², which are known to be homologous to TGF¹³, and residues 45–85 of a 140-residue polypeptide encoded by VV⁶ (Fig. 1). Fifteen residues of the VV polypeptide match residues in rTGF and, after insertion of a single gap, the viral polypeptide shares 19 residues with both mEGF and hEGF; a gap must be inserted in the same position in TGF to align it with EGF. The viral protein is thus as similar to EGF as is rTGF, which has 16 residues in common with mEGF and 20 with hEGF. The statistical significance of the homology can be determined as follows. Of the 16 residues of mEGF which are conserved in rTGF, 13

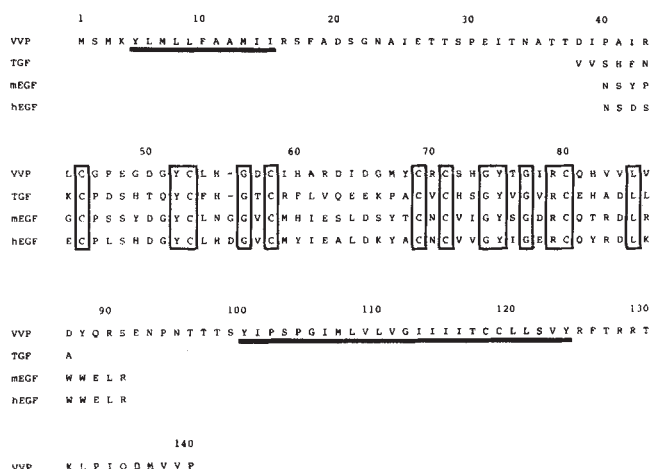


Fig. 1 Alignment of the VV polypeptide (VVP), rTGF, mEGF and hEGF. The sequences are shown in their entirety, numbered from the N-terminus of VVP. Residues conserved in all four sequences are boxed. Putative signal and transmembranous sequences are underlined. A, Ala; R, Arg; N, Asn; D, Asp; C, Cys; Q, Gln; E, Glu; G, Gly; H, His; I, Ile; L, Leu; K, Lys; M, Met; F, Phe; P, Pro; S, Ser; T, Thr; W, Trp; Y, Tyr; V, Val.

are conserved in the VV polypeptide, whereas of the remaining 37 residues of mEGF, only six are conserved in the VV polypeptide. Fisher's exact test shows that the probability of this being due to chance is less than 0.0001. In addition to EGF, the EGF precursor encodes eight sequences that resemble EGF in their pattern of cysteine residues and their predicted folding patterns^{7,8,14}; EGF-related sequences are also present in the low-density lipoprotein receptor¹⁵ and the blood coagulation zymogens factor IX, factor X and protein C (ref. 14). It thus appears that these sequences and EGF diverged from a common ancestor¹⁴. However, at least five gaps must be inserted to align the VV polypeptide with any of these sequences, and the VV polypeptide is most closely related to EGF and TGF.

The EGF-homologous region of the VV polypeptide differs from the rest of the molecule in its amino acid composition, particularly in that it contains 6 of the 8 cysteine residues and 7 of the 10 glycine residues. These residues may have a structural role. The six cysteine residues correspond to the six cysteine residues that, in EGF, form three disulphide bridges, resulting in three tight loops in the polypeptide chain¹⁶. Three of the glycine residues are conserved in EGF and TGF, and together with the four aspartic acid residues and the single proline residue present in this region of the VV polypeptide, they may allow the chain to fold back on itself within the right constraints imposed by the disulphide bridges^{14,16,17}. Two regions of particularly high homology are evident; these are residues 50–56, all of which are identical to residues in the corresponding positions in either EGF or TGF, and residues 73–82, seven of which are conserved in EGF or TGF. We therefore conclude that the VV polypeptide probably resembles EGF and TGF not only in its amino acid sequence, but also in its pattern of disulphide bridges and thus in its three-dimensional structure.

Further analysis of the sequence of the viral protein revealed the presence of stretches of uncharged and hydrophobic residues near the N-terminus between residues 5 and 15 and near the C-terminus between residues 100 and 124. This arrangement of hydrophobic regions is similar to that seen in many integral membrane glycoproteins, which have an N-terminal hydrophobic signal sequence and a C-terminal transmembranous sequence. The transmembranous sequence is followed commonly by several basic residues and in the VV polypeptide, residues 125, 128 and 129 are arginine. The EGF precursor itself has signal and transmembranous sequences; it has been suggested that it exists on the surface of the cells of its origin anchored by the hydrophobic segment, EGF being released by

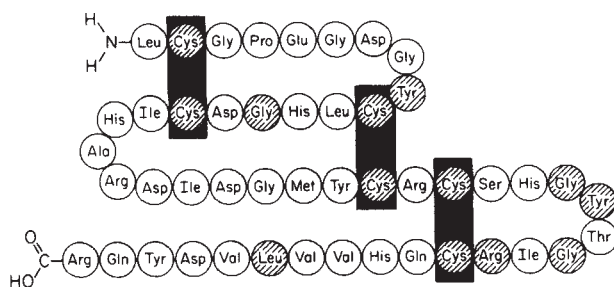


Fig. 2 Proposed structure of the VV polypeptide that would be produced by cleavage of the precursor at Arg 43 and Arg 90. Disulphide bonds (boxed) are based on those of EGF. Residues that are identical in the VV polypeptide, mEGF, hEGF and rTGF are cross-hatched.

proteolytic cleavage¹⁴. The transmembranous peptides of both the EGF precursor and the VV polypeptide are located ~20 residues from the end of the EGF-like sequence; cleavage of the viral polypeptide at Arg 43 and Arg 90 would lead to release of a soluble polypeptide of 47 residues (Fig. 2).

As the VV polypeptide is structurally as similar to EGF as is TGF, we believe that, like TGF, it might be able to bind the EGF receptor and act as a growth factor. This suggests several possible roles for the VV polypeptide. If it is located on the surface of the virion, it might mediate binding of the virus to cells expressing the EGF receptor and also stimulate the host cell. If, on the other hand, a soluble EGF-like peptide is released from VV-infected cells, neighbouring cells might be stimulated. It is intriguing to speculate that involvement of the EGF receptor in VV infection might contribute towards the propensity of the virus to infect epidermal cells, while production of EGF-like growth factors might account for the cellular proliferation associated with poxviruses both *in vivo*^{9–11} and *in vitro*^{18–20}.

In conclusion, the structural homology between the VV polypeptide and EGF and TGF indicates clearly an evolutionary relationship. Whether a functional relationship exists remains to be confirmed experimentally. Nevertheless, our observation, together with the many reports of the capture of cellular genes by retroviruses, including genes for platelet-derived growth factor²¹ and EGF receptor²², suggest that viral capture of cellular genes coding for growth factors and receptors might be more common than is realized generally and might not be restricted to the retroviruses.

Note added in proof: Blomquist *et al.*²³ have recently reported findings similar to those described here.

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Suppression of the normal mouse *c-myc* oncogene in human lymphoma cells

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In Burkitt's lymphoma, which carries the t(8;14) chromosome translocation, the *c-myc* oncogene normally located on band q24 of human chromosome 8 (refs 1–3) translocates to the heavy-chain locus on chromosome 14 (refs 1, 4, 5); this results in transcriptional deregulation of the translocated *c-myc* oncogene, which is transcribed constitutively at elevated levels^{5–9}, while the normal *c-myc* oncogene on the uninvolved chromosome 8 is either silent^{6–11} or expressed at very low levels (A.ar-R. and C.M.C., unpublished results). We have now introduced the active *c-myc* oncogene of proliferating mouse spleen cells into human lymphoma cells carrying the t(8;14) chromosome translocation by hybridization, and have examined the hybrids for expression of the human and murine *c-myc* oncogene. The results of this analysis, reported here, indicate that the active mouse *myc* gene is shut off at the transcriptional level in the human lymphoma cells, implying that human B cells at the stage of differentiation of lymphoma cells used in this study are nonpermissive for normal *c-myc* transcription.

Somatic cell hybrids between simian virus 40 (SV40)-transformed human cells and cells derived directly from mice segregate human chromosomes, but preferentially retain the human chromosome carrying the SV40 genome^{12–14}; such hybrids are transformed *in vitro* and tumorigenic *in vivo*¹³. As hybridization of cells derived directly from mice with SV40-transformed human cells results in the selection of hybrids containing the human chromosome carrying the SV40 genome and these hybrids are transformed and tumorigenic¹³, we hybridized mouse spleen cells with lymphoma cells carrying the t(8;14) translocation to determine whether such hybrids would segregate human chromosomes, preferentially retain the human 14q⁺ chromosome, and exhibit a transformed phenotype *in vitro* and tumorigenic phenotype *in vivo*.

Thus, we hybridized mouse spleen cells which had been stimulated to proliferate by a B-cell mitogen, bacterial lipopolysaccharide (LPS), with SK-DHL2B lymphoma cells carrying a



Fig. 1 G-11 staining of metaphase chromosomes from hybrid cell SKM 6.2.4 SC1 6. The pale chromosomes are human; the dark chromosomes are of murine origin. The hybrid contains 10 mouse chromosomes. Chromosome analysis was performed on an air-dried chromosome preparation banded with trypsin-Giemsa by standard methods⁵. After examination of at least 25 metaphases from each hybrid cell line, selected metaphases were destained and restained by the G-11 technique⁵ to distinguish the murine from the human chromosomes.

reciprocal t(8;14) chromosome translocation¹⁵. Activation of the translocated *c-myc* oncogene in this cell line has been reported previously¹⁶. The resulting hybrid cells segregated mouse chromosomes rather than human chromosomes (Fig. 1); 5–10 mouse chromosomes were retained by different hybrid clones.

Table 1 shows that the mouse × SK-DHL2B hybrids retained the phenotype of the parental human lymphoma cells. We examined these hybrid cells for the presence of the mouse *c-myc* gene to determine whether the murine chromosome 15 had been retained^{17,18}. Figure 2 shows that the hybrids retained both the human and the murine *c-myc* oncogene. Therefore, these hybrids provided the opportunity to study the regulation of expression of the previously transcriptionally active murine *c-myc* oncogene in a human lymphoma cell carrying a t(8;14) translocation.

A probe specific for exon 1 of the human *c-myc* gene detected normal (2.2–2.4-kilobase, kb) *c-myc* transcripts only in a Burkitt's lymphoma cell line (Daudi) carrying an intact translocated *c-myc* gene¹ (Fig. 3a), but neither in the SK-DHL2B cells nor in ST486 Burkitt's lymphoma cells carrying rearranged translocated *c-myc* genes, indicating that the normal human *c-myc* gene on the uninvolved chromosome 8 is transcriptionally silent in SK-DHL2B and ST486 cells. The upper band in Fig. 3a is due to cross-hybridization of the *myc* exon 1 probe with 28S ribosomal RNA¹⁹. As shown previously, the separated first exon of the *c-myc* oncogene on the 8q⁺ chromosome is transcribed at high levels in the ST486 Burkitt lymphoma cells⁷ (Fig.

Table 1 Phenotype of parental and hybrid cells

Parental cell line and hybrids	Mouse isozymes		Mouse <i>c-myc</i>	Human surface markers*		Immunoglobulins		Level of <i>c-myc</i> transcription	
	NP	LDH		B-532	B1	Human	Mouse	Human	Mouse
SK-DHL2B	–	–	–	–	+++	IgM	–	+++	–
Hybrid SKM 6.2.4 SC1 1	+	–	+	–	+++	IgM	–	+++	–
Hybrid SKM 6.2.4 SC1 3	+	–	+	–	+++	IgM	–	+++	–
Hybrid SKM 6.2.4 SC1 6	+	–	+	–	+++	IgM	–	+++	–
Mouse spleen cells (with 48 h of LPS stimulation)	+	+	+	–	–	ND	–	–	++

NP, nucleoside phosphorylase; LDH, lactic dehydrogenase; ND, not determined.

* Monoclonal antibodies against human B-cell antigens were used. The anti-B-532 antibodies reacted²⁴ with the human lymphoblastoid cell lines tested, but not with several Burkitt lymphoma cell lines (Daudi, CA46, BL-2 and J1). These antibodies are human-specific. The anti-B1 antibodies²⁵ reacted with lymphoblastoid and Burkitt lymphoma cell lines.

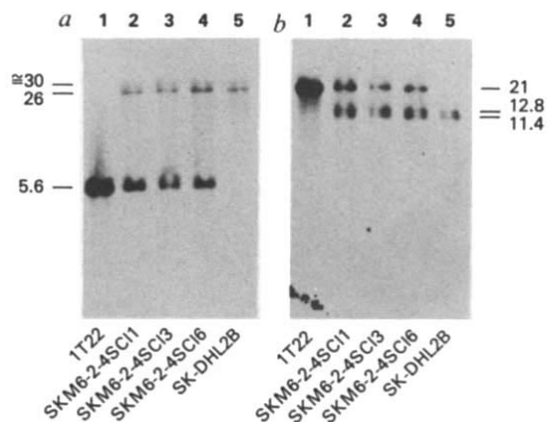


Fig. 2 Southern blot analysis of *Bam*HI (a) and *Eco*RI (b) digested DNAs from hybrids and parental cells. The DNA on the filter was hybridized with the pMc-myc54 mouse *myc* cDNA probe⁶ to detect the presence of mouse chromosome 15 on which the *c-myc* gene is located. As this probe cross-hybridizes with the human *c-myc* gene, we detected fragments corresponding to the mouse *c-myc* sequences (a and b, lanes 2, 3 and 4 respectively) as well as the restriction fragments corresponding to the normal and rearranged *c-myc* gene derived from the human parental cell line in the DNA of hybrid cells. In a and b, lane 1, the DNA is from a mouse fibroblast cell line (IT22) which has a normal *c-myc* gene. Lane 5, DNA from the human parental cell line SK-DHL2B. DNAs (10 µg per sample) were digested with restriction enzymes, electrophoresed in an agarose gel and transferred to nitrocellulose filters. Hybridization conditions: 6×SSC, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin (BSA), 0.2% Ficoll, 0.5% SDS, and 100 µg ml⁻¹ of sonicated salmon sperm DNA containing 10⁶ c.p.m. ml⁻¹ of nick-translated ³²P probe (specific activity 10⁸ c.p.m. µg⁻¹). After hybridization, filters were washed with 0.1×SSC and 0.5% SDS and autoradiographed at -70 °C. Lambda phage DNA cut with *Hind*III was run in the same gel to determine the restriction fragment sizes.

3a, lane 2; 0.7–0.9-kb transcripts). However, the cDNA *myc* probe specific for exons 2 and 3 of the *c-myc* oncogene detects *myc* transcripts in all three lymphoma cell lines carrying the t(8;14) translocation (Fig. 3b).

We have shown previously that S₁ nuclease protection experiments can detect the expression of human or murine *c-myc* transcripts by using a human or a mouse *c-myc* cDNA S₁ probe respectively^{6–9}. The human probe detected the expression of the human *myc* transcripts in three hybrid clones carrying both the mouse and human *c-myc* genes and in the SK-DHL2B parental cells (Fig. 4a); the mouse cDNA probe detected murine *myc* transcripts in mouse spleen cells before (Fig. 4b, lane 6) and after (lane 7) mitogenic stimulation with LPS as well as in P3Bu mouse myeloma cells (lane 8). However, no protection of the mouse *myc* cDNA was detected using RNA from the hybrid clones (Fig. 4b, lanes 3–5). Thus, these hybrids, which are phenotypically similar to the Burkitt lymphoma parental cells, do not express the germ-line murine *c-myc* gene. It is unlikely that the lack of expression of murine *c-myc* in these human-mouse hybrids is due to lack of a species-specific factor in human-mouse hybrids because expression of both human and murine *c-myc* genes is observed in hybrids between murine 3T3 fibroblasts and human lymphoblastoid cells and in hybrids between mouse and human fibroblasts (data not shown).

Another possible explanation for the lack of expression of the normal mouse *c-myc* gene in the mouse spleen × SK-DHL2B hybrids is that the spleen cells which were able to fuse with the human lymphoma cells were themselves at a stage of differentiation at which *myc* is inactive; this seems unlikely, however, as B-cell hybridization occurs preferentially between proliferating B cells²⁰ and it has been shown that B cells induced to proliferate express the *c-myc* oncogene²¹.

Recently, Rabbitts *et al.*²² have shown that normal *c-myc* transcripts are expressed in the Burkitt lymphoma cell line Raji

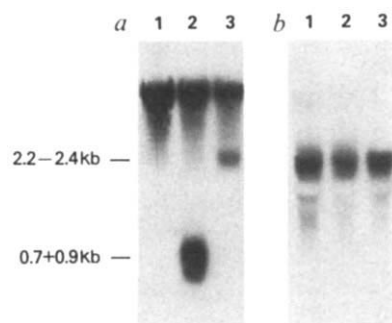


Fig. 3 Northern blot analysis of RNA from the human cell line SK-DHL2B (lane 1) and two Burkitt lymphoma cell lines, ST486 and Daudi (lanes 2 and 3, respectively). The RNAs were hybridized as follows: a, with the 5' exon probe of the human *c-myc* gene⁷; and b, with pRyc7.4 probe, specific for exons 2 and 3 (ref. 6). All three lymphoma cell lines showed the 2.4–2.2-kb *c-myc* transcript with the pRyc7.4 probe (b); the same transcripts were detected only in Daudi cells using the 5' exon probe (a, lane 3). In ST486 cells only the 0.9- and 0.7-kb transcripts derived from the decapitated first exon were detected using the exon 1 probe (a, lane 2); no *myc* transcripts were detected in SK-DHL2B with this probe (a, lane 1), indicating that neither the normal *c-myc* gene nor the decapitated first exon was transcribed in this cell line.

Methods. Cytoplasmic RNA was extracted, glyoxylated and 20 µg of each sample were separated by 1.0% agarose gel electrophoresis and transferred to nitrocellulose filters⁷. The filters were hybridized at 37 °C for 30 h in 40% formamide, 10 mM phosphate buffer pH 6.5, 4×SSC, 1×Denhardt's, 200 µg ml⁻¹ BSA, 10% dextran sulphate and 5 µg ml⁻¹ of a ³²P-labelled nick-translated probe (specific activity 10⁸ c.p.m. µg⁻¹). Hybridized filters were then washed with 2×SSC, 0.1% SDS at room temperature, then at 55 °C with 0.1×SSC, 0.1% SDS, and autoradiographed at -70 °C.

and have speculated that the expression of the normal *c-myc* allele results from mutations of the translocated *c-myc* gene, yielding an altered *c-myc* protein that is unable to autoregulate normal *c-myc* transcription. This interpretation is not supported by the results of Tonegawa and his associates, however, who have observed co-expression of normal and translocated *myc* transcripts in human lymphoblastoid cells transfected with a cloned translocated *c-myc* gene (S. Tonegawa, personal communication) from the same SK-DHL (Manca) line studied here. As SK-DHL cells express only the translocated *c-myc* gene (Fig. 3), if the normal *c-myc* oncogene were autoregulated by the translocated *c-myc* oncogene, co-expression of the normal and the translocated *myc* gene should not be observed in transfectants.

We have also observed co-expression of translocated and normal *c-myc* genes in lymphoblastoid-like hybrids between Daudi and human lymphoblastoid cells²³. Thus, co-expression of normal and rearranged (SK-DHL-derived; S. Tonegawa, personal communication) or translocated (Daudi-derived; C.M.C. *et al.*²³) *c-myc* genes in lymphoblastoid cells seems to rule out direct *c-myc* autoregulation. We have confirmed the observation of Rabbitts *et al.*²² that the untranslocated *c-myc* gene is transcribed in Raji cells, however, by performing S₁-nuclease protection experiments using a *c-myc* exon 1 probe to quantitate the levels of *c-myc* transcripts of the translocated versus the untranslocated *c-myc* oncogene, we found that the level of normal *c-myc* transcripts is less than 5% of the level of transcripts of the translocated *c-myc* gene (K. Nishikura and C.M.C., unpublished results). We have also found co-expression of transcripts of the normal and the translocated *c-myc* gene in CA46 Burkitt lymphoma cells, in which the level of the normal *myc* transcripts is less than 2% of the level of transcripts of the translocated *c-myc* oncogene (data not shown). Thus, most, but not all, Burkitt lymphomas suppress completely the expression of the normal *c-myc* oncogene. A possible explanation for variation in the level of suppression of the normal *c-myc* gene in Burkitt lymphomas is that different Burkitt lymphoma cells may represent cells at different stages of B-cell differentiation.

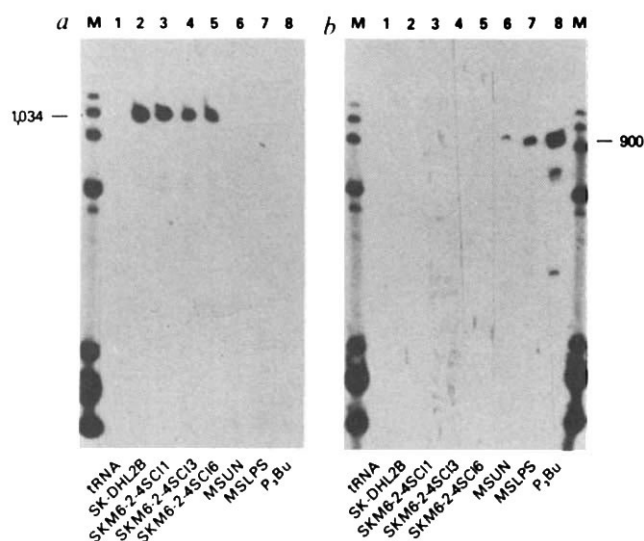


Fig. 4 S_1 nuclease analysis of *c-myc* RNA in the mouse spleen/human SK-DHL2B hybrid cells. Due to the difference in nucleotide sequence, we were able to distinguish between the murine and human *c-myc* transcripts using two different cDNA probes^{6,8,9}. In *a*, a human *c-myc* cDNA probe (Ryc7.4) specific for exons 2 and 3 of the *c-myc* gene was used⁶. The expected S_1 -nuclease-resistant product using this probe is 1,034 base pairs (bp) long. Hybrids SKM 6.2.4 SC1 1, 3 and 6 (lanes 3, 4 and 5, respectively) all expressed the same levels of human *c-myc* transcripts as the parental cell line SK-DHL2B (lane 2). In *b*, the same RNAs were analysed with a mouse *c-myc* probe. The expected S_1 -nuclease-resistant product, which is 900 bp long, was detected in mouse spleen cells before (lane 6) and after (lane 7) mitogenic stimulation as well as in mouse myeloma P3Bu (lane 8). No protected fragments were detected in the hybrid cells (lanes 3–5). **Methods.** RNA was prepared by phenol extraction in the presence of 1% SDS from Nonidet P-40 cell lysates after separation of the nuclear fraction. RNA (20 μ g per sample) was hybridized with a [³²P]-end-labelled DNA probe in 80% formamide for 12 h. After digestion of the hybrid product with 3,000 IU of S_1 nuclease (Boehringer) at 37 °C for 60 min, the protected fragments were analysed in a 7 M urea, 4% polyacrylamide gel. Φ X174 *Hae*III-digested DNA was used as a size marker.

To conclude, hybrids between the SK-DHL2B Burkitt lymphoma (in which the normal *c-myc* gene is transcriptionally silent) and LPS-stimulated mouse spleen cells (in which the murine *c-myc* gene is actively transcribed) retain the phenotype of the Burkitt lymphoma parental cells, segregate mouse chromosomes and suppress expression of the normal murine *c-myc* gene. It is unlikely that the suppression of the murine *c-myc* gene in these hybrids is due to lack of some mouse-specific factor, or to selection of hybrid cells derived from non-proliferating mouse parental cells, nor is suppression mediated by an autoregulatory function of the gene product of the translocated *c-myc* locus. Instead, we favour the theory that Burkitt's lymphoma cells represent a state of B-cell differentiation in which a normal *c-myc* gene is normally silent, that is, Burkitt lymphoma cells and their postulated normal counterpart in the B-cell lineage are nonpermissive (or nearly so) for expression of an intact *c-myc* gene.

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Detection of single base substitutions in total genomic DNA

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Certain single base substitutions causing genetic diseases^{1–4} or resulting in polymorphisms linked to mutant alleles^{5–7}, alter a restriction enzyme cleavage site and can therefore be detected in total genomic DNA using DNA blots. Many base substitutions do not lead to an altered restriction site, but these can be detected using synthetic oligonucleotides as hybridization probes if the DNA sequence surrounding the base substitution is known^{8,9}. In the case of β -thalassaemia, where 22 different single base mutations have been identified¹⁰, a large number of probes would be required for diagnosis. An approach which was used to detect mutations in viral DNA involves the S_1 nuclease treatment of heteroduplexes formed between wild-type and mutant DNA¹¹. Although certain single base mismatches are cleaved by S_1 nuclease (ref. 11 and T. Shenk, personal communication), many other mismatches examined by this procedure are not cleaved (B. Seed, personal communication; R.M.M., unpublished data). Heteroduplexes between mutant and wild-type subgenomic fragments of double-stranded reovirus RNA migrate slower than the corresponding homoduplexes in polyacrylamide gels containing 7 M urea¹², but it is not known whether this method is applicable to DNA heteroduplexes containing single base mismatches. Here we describe a procedure that involves the electrophoretic separation of DNA heteroduplexes in a well-characterized gel system¹³. We show that four different human β -thalassaemia alleles with known single base mutations can be detected with as little as 5 μ g of total genomic DNA. The method should be useful in the localization and diagnosis of mutations associated with genetic diseases.

Single base substitutions can be detected in bacteriophage λ DNA¹³ and in cloned DNA homoduplexes (ref. 14 and R.M.M. *et al.*, manuscript in preparation) by electrophoresis through a polyacrylamide gel containing a gradient of DNA denaturants parallel to the direction of electrophoresis. The separation is based on differences in the melting behaviour of the wild-type and mutant DNA molecules (see ref. 14 for discussion). We have found that these differences in DNA melting are increased in heteroduplexes containing a single base mismatch (manu-

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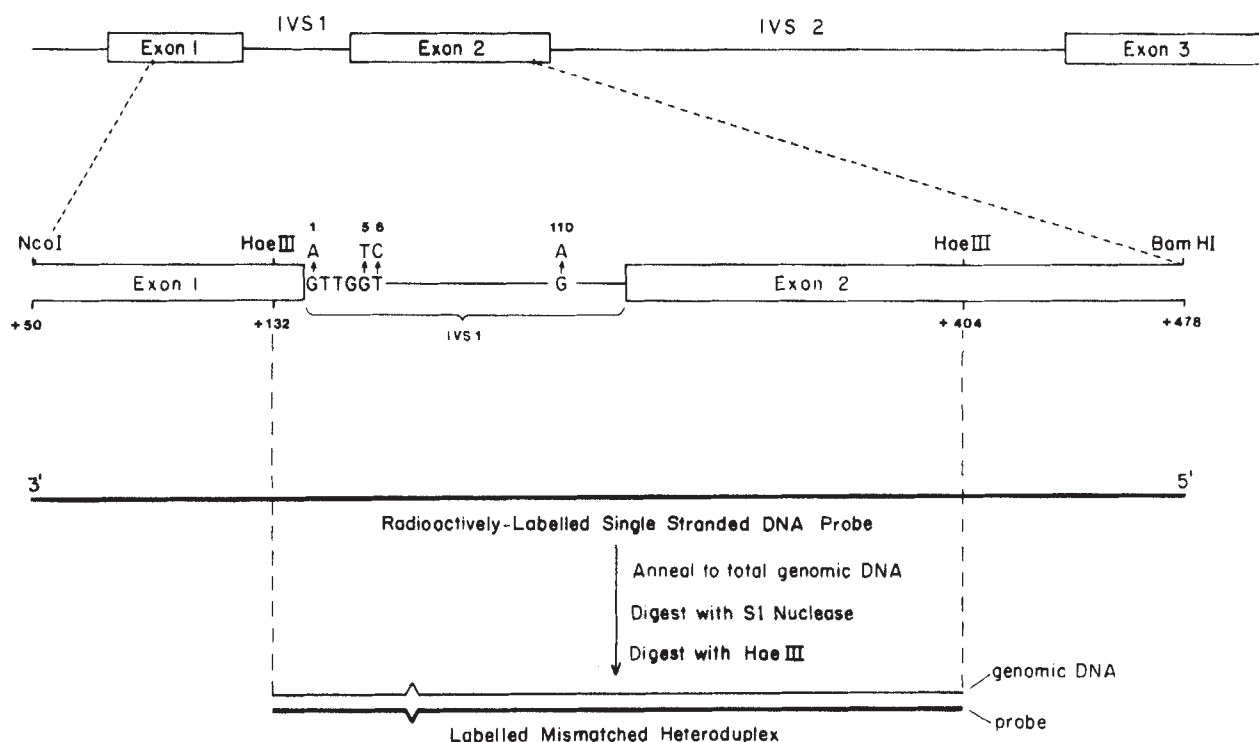


Fig. 1 Schematic diagram showing the location of four β -thalassaemia mutations in the human β -globin gene. The entire gene, which contains three exons and two intervening sequences (IVS), is shown at the top of the figure. Each of the single base substitutions, numbered according to their positions within IVS-1, are indicated in the expansion of the IVS-1 region shown below the gene. The IVS-1 positions 1 and 6 alleles were described by Orkin *et al.*⁷, and IVS-1 110 was originally reported by Westaway and Williamson²⁰ and Spritz *et al.*²¹. The IVS-1 position 5 mutation was localized by Atweh *et al.*¹⁶. The DNA fragment used to prepare a single-stranded hybridization probe extends from an NcoI site at position +50 in exon 1 to a BamHI site at position +478 in exon 2²². The probe, complementary to the sense strand of the globin gene, is shown at the bottom of the figure.

Methods. ^{32}P -labelled probe was prepared as follows: The NcoI to BamHI fragment was inserted into the plasmid pMH β 8 (R.M.M., unpublished) which carries a phage M13 origin of DNA replication²³. The resulting plasmid (pGCH β 1) was used to prepare single-stranded DNA template by infecting the F' strain (LE392 F') carrying the plasmid with the M13 phage ϕ 1²³. A mixture of single-stranded pGCH β 1 and helper ϕ 1 DNA was then recovered as described previously²³. ^{32}P -labelled single-stranded DNA probe was synthesized by extension of a synthetic oligonucleotide primer homologous to the plasmid sequence adjacent to the NcoI to BamHI insert in pGCH β 1. 10 pmol primer was mixed with 1 pmol single-stranded DNA template at 40 °C for 10 min, followed by incubation with 5 units of Klenow fragment polymerase (New England Biolabs) for 1 h at 22 °C. The 30- μ l reaction mixture contained 100 μ Ci [α - ^{32}P]dATP ($\sim 1,000$ Ci mmol⁻¹), 150 μ M dGTP, dCTP and dTTP in 6 mM Tris-HCl (pH 7.4), 50 mM NaCl, 6 mM MgCl₂ and 2 mM dithiothreitol²⁴. The reaction mixture was incubated at 22 °C for 1 h and the reaction was terminated by heating at 70 °C for 5 min. The probe was truncated by digestion with NcoI and purified by electrophoresis on a 4% polyacrylamide/7 M urea gel. Trace amounts of contaminating double-stranded material were removed by treating the probe preparation with restriction enzymes that do not cleave single-stranded DNA (AccI, XhoII, MboII, MnlI, HphI and AluI). The enzymes were inactivated by incubation at 75 °C for 5 min and the probe recovered by ethanol precipitation. This procedure yielded ~ 100 ng of single-stranded DNA probe with a specific activity of 2×10^9 c.p.m. μ g⁻¹.

script in preparation). Thus, the separation of heteroduplexes and wild-type homoduplexes is significantly greater than the separation of wild-type and mutant homoduplexes on denaturing gradient gels.

We have examined four well-characterized β -thalassaemia mutations, each one resulting from a different single base substitution in the human β -globin gene. These four mutations are localized in the first intervening sequence (IVS-1) of the β -globin gene^{15,16}, and each has been shown to cause defects in RNA splicing (refs 15, 17 and B. Forget, personal communication). DNA samples containing the β -thalassaemia mutations located at IVS-1 positions 1, 5, 6 and 110 (Fig. 1) were digested with restriction enzymes that cleave outside of the fragment of interest, denatured by boiling and mixed with a 6,400-fold excess of a labelled single-stranded DNA probe. After annealing, the hybridization mix was digested with S₁ nuclease to remove the unreacted probe and genomic DNA sequences. The duplex DNA was then digested with the restriction enzyme HaeIII which generates a 272-base pair duplex DNA fragment containing IVS-1. This DNA fragment was then subjected to electrophoresis on a denaturing gradient polyacrylamide gel, followed by autoradiography.

Initially, we examined duplexes formed between the labelled probe and cloned DNA fragments containing the β -

thalassaemia mutations. As shown in Fig. 2a, each of the mutant heteroduplexes clearly separates from the wild-type homoduplexes when electrophoresed through a denaturing gradient gel (lanes 1-5). In the cases shown here, the separation between mutant and wild-type DNA duplexes is from 1 to 4 cm, depending on the mutation examined. We next demonstrated that the separation observed with the cloned DNA fragments can also be observed in total genomic DNA from β -thalassaemia patients. As shown in Fig. 2b (lane 1), the final gradient position of the duplex formed between the probe and normal human genomic DNA is the same as that observed with the corresponding duplex of cloned DNA (Fig. 2a, lane 1). Similarly, two bands were observed with a DNA sample isolated from a cell line derived from a patient heterozygous for the IVS-1 position 1 and IVS-1 position 6 β -thalassaemia alleles (Fig. 2b, lane 2). The positions of these bands are the same as those of the corresponding cloned DNA samples (Fig. 2a, lanes 2 and 3). The same is true for the IVS-1 position 110 and IVS-1 position 5 alleles (Fig. 2b, lanes 3 and 4). The DNA sample containing the IVS-1 position 5 allele was obtained from a patient with two β -thalassaemia alleles. The second allele contains a single base substitution at IVS-2 position 1, which lies outside the region detected by the IVS-1 probe¹⁷. Since IVS-1 in the second allele is wild type, the gel position of the duplex formed between the

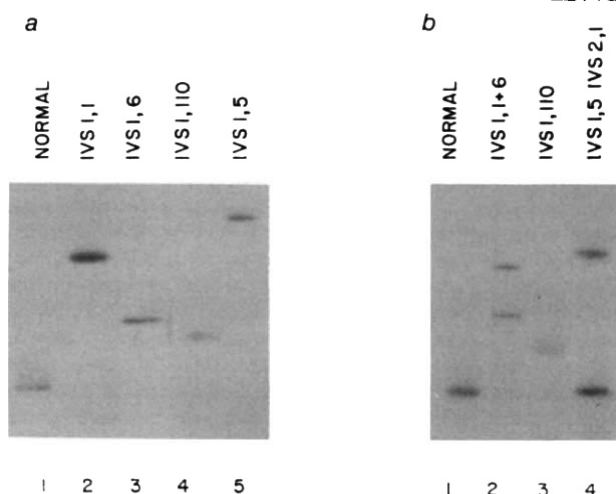


Fig. 2 Denaturing gradient gel electrophoresis of heteroduplexes between wild-type and mutant β -globin gene fragments. *a*, An autoradiogram showing the analysis of duplexes formed between the labelled single-stranded DNA probe and cloned DNA fragments from the normal human β -globin gene (lane 1), the IVS-1 position 1 allele (lane 2), the IVS-1 position 6 allele (lane 3), the IVS-1 position 110 allele (lane 4), and the IVS-1 position 5 allele (lane 5). *b*, An autoradiogram showing the analysis of duplexes formed between the labelled single-stranded DNA probe and genomic DNA from an individual with normal β -globin genes (lane 1), a patient heterozygous for the IVS-1 position 1 and IVS-1 position 6 alleles (lane 2), a patient homozygous for the IVS-1 position 110 allele (lane 3) and a patient heterozygous for the IVS-1 position 5 and IVS-2 position 1 alleles (lane 4).

Methods. 10 μ g of each genomic DNA sample was digested with *Nco*I and *Bam*HI and mixed with 0.05 pmol (5 pg, $\sim 10^7$ c.p.m.) of single-stranded DNA probe in 500 mM NaCl in a final volume of 20 μ l. The samples were placed in a boiling water bath for 5 min and then annealed for 4 h at 50 $^{\circ}$ C. The samples were chilled on ice and mixed with 300 μ l of a solution containing 900 U ml $^{-1}$ *S*₁ nuclease (Sigma) in 30 mM sodium acetate (pH 4.4), 280 mM NaCl, 5 mM ZnCl₂ and 10 μ g ml $^{-1}$ denatured salmon sperm DNA carrier²⁵. The reaction mix was incubated for 1 h at 18 $^{\circ}$ C, and *S*₁ digestion terminated by the addition of 75 μ l 2.5 M ammonium acetate, 50 mM EDTA and 20 μ g transfer RNA. The undigested duplex DNA was precipitated with isopropanol and then digested with restriction enzyme *Hae*III, which cleaves the *Nco*I to *Bam*HI fragment to produce a 272-base pair *Hae*III fragment carrying the IVS-1 mutations (Fig. 1). The DNA was then ethanol-precipitated and one half of the sample loaded onto a 17 cm $\times$ 17 cm, 0.6 mm-thick 14% polyacrylamide gel containing a 30–60% denaturant gradient parallel to the direction of electrophoresis (100% denaturant = 7 M urea and 40% formamide; acrylamide/bisacrylamide = 37.5/1). Gradient gels were formed by mixing stock solutions containing 60 and 30% denaturant (each denaturant solution contains a constant concentration of 14% acrylamide and TAE buffer) in a two-chambered gradient maker and dripping the solution from the top of the gel as it mixed. The gel was run at 150 V for 10.5 h submerged in an aquarium containing TAE buffer (40 mM Tris-acetate, pH 7.4, 20 mM sodium acetate, and 1 mM EDTA) that was maintained at a constant temperature of 60 $^{\circ}$ C by a heater, thermostat and stirrer. After electrophoresis the gel was directly subjected to autoradiography at -70° C with an intensifying screen. The cloned DNA samples were treated in the same manner as the genomic DNA, except that 0.5 μ g of plasmid DNA was used and 0.01% of the sample was loaded on the gel.

probe and this allele is indistinguishable from that observed with the normal β -globin gene (lane 1 in Fig. 2*a*, *b*). These results indicate that it is possible to determine unambiguously whether an individual is homozygous or heterozygous for a particular β -thalassaemia allele.

Our protocol involves the treatment of the hybridization mixture with *S*₁ nuclease to remove the unassociated probe and genomic DNA. As noted above, single base mismatches are rarely cleaved by *S*₁ nuclease—we have not observed cleavage in any of a large number of mismatches examined. In the absence

of *S*₁ nuclease digestion, considerable background from the unreacted probe is observed, making it difficult to detect the globin gene fragments. The *S*₁ nuclease step can be avoided if a smaller amount of probe is used, but longer hybridization times are required. If the mutation of interest is a deletion or addition it is probable that *S*₁ will cleave the heteroduplex to generate two smaller fragments (ref. 12 and B. Seed, personal communication). Thus, the presence of the mutation will be indicated by the disappearance of the heteroduplex DNA and the appearance of two smaller DNA fragments.

Although the genomic DNA heteroduplex method has several advantages over procedures used currently for detecting mutations in total genomic DNA, some single base substitutions will not lead to a shift in gradient position of the heteroduplex in denaturing gradient gels. For example, a heteroduplex between the wild-type probe and the thalassaemia allele with a G $\rightarrow$ A transition at IVS-2, position 1 (ref. 17) does not separate from the wild-type homoduplex (data not shown). The failure of certain mutant β -globin alleles to separate from wild-type DNA fragments is a property of the melting behaviour of DNA. As duplex DNA moves through the concentration gradient of denaturants in the polyacrylamide gel, melting occurs in discrete domains. If the mutation is located in the highest-temperature melting domain, or if the fragment melts as a single domain, no shift is observed. In some cases this problem can be overcome by choosing a different restriction enzyme which would place the mutation in a fragment with a higher melting domain. In any case, studies of the melting behaviour of many DNA fragments indicate that between 25 and 40% of all possible single base substitution mutations in most genes should be detectable by this method. Work is in progress to modify the procedure so that all single base substitutions can be detected.

Although the genomic heteroduplex method reported here cannot be used to identify all single base substitutions, it should be applicable to a variety of problems in molecular genetics. For example, a similar procedure has been used to detect different alleles or somatic variants of immunoglobulin genes (J. Scott, L.S.L. and G. P. Smith, personal communication). In addition to the detection of well-characterized mutations associated with a genetic disease, as shown here, the genomic heteroduplex procedure should facilitate the identification of new sequence polymorphisms for human genetic linkage studies^{18,19}. In contrast to current methods used to detect new polymorphic variants, this procedure obviates the need to screen each DNA sample with a large number of different restriction endonucleases. Finally, the genomic heteroduplex method should be useful for localizing mutations in a variety of experimental systems in which mutants can be phenotypically selected.

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Intron-dependent evolution of chicken glyceraldehyde phosphate dehydrogenase gene

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The function of introns in the evolution of genes can be explained in at least two ways: either introns appeared late in evolution and therefore could not have participated in the construction of primordial genes, or RNA splicing and introns existed in the earliest organisms but were lost during the evolution of the modern prokaryotes. The latter alternative allows the possibility of intron participation in the formation of primordial genes before the divergence of modern prokaryotes and eukaryotes^{1,2}. Blake³ suggested that evidence for intron-facilitated evolution of a gene might be found by comparing the borders of functional protein domains with the placement of introns. We therefore examined glyceraldehyde phosphate dehydrogenase (GAPDH), a glycolytic enzyme, because it is the first protein for which the following data are available: (1) X-ray crystallographic studies demonstrating structurally independent protein 'domains' which were highly conserved during the divergence of prokaryotes and eukaryotes^{4,5}; and (2) a study of genomic organization which mapped introns in the gene⁶. Sequencing of the chicken GAPDH gene revealed 11 introns. We report here that sites of three of the introns (IV, VI and XI) correspond closely with the borders of the NAD-binding, catalytic and helical tail domains of the enzyme, supporting the hypothesis that introns did have a role in the evolution of primitive genes. In addition, other biochemical and structural data were used to construct a model of the intron-mediated assembly of the GAPDH gene that explains the existence of 10 introns.

GAPDH is present in both prokaryotes and eukaryotes and is highly conserved across great evolutionary distances (see Fig. 1). GAPDH amino acid sequences from seven species (eight genes) were compared for homology as described in Fig. 1 legend. The proteins from all the species examined are nearly equal in length (332-334 amino acids) and the numerous amino

acids that are wholly conserved among all eight variants are scattered throughout the protein, suggesting that these variants arose from a single ancestral gene and that no exon addition, deletion or rearrangement occurred after the divergence of the modern prokaryotes and eukaryotes. Therefore, the proteins compared in Fig. 1 are similar not only to each other, but also to the primordial GAPDH protein. Thus, many structural clues to the ontogeny of the ancestral GAPDH gene may remain relatively undisturbed in an organism that has intervening sequences in its DNA.

Conceptually, intervening sequences could have had two broad roles in the formation of modern genes: associative^{2,7} (type A) and divisive (type B). An associative intron would come into being when two unrelated exons were joined by an unequal recombination of two genes. The introns would render this gene fusion event insensitive to a shift in the reading frame, and allow a novel unequal recombination to succeed within a target site of thousands of nucleotides instead of one or just a few^{2,7}. Hence, a 'type A' intron would 'function' evolutionarily by increasing the frequency of successful shuffling of gene segments. If the early organisms contained such introns (and the necessary RNA splicing machinery), they also might have tolerated random insertions of DNA (bearing rudimentary splicing signals) into previously intact genes. Such 'type B' introns could arise by aberrant recombination, retrovirus or transposon integration, or by retrovirus-mediated insertion of cellular mRNA sequences.

If introns arose in this manner during the evolutionary assembly of a gene, some evidence for these events might still be found in the structures of the protein and gene: for example, if an associative intron facilitated the original assembly of a crystallographically defined domain, one might expect to find that two or more amino acid residues which are essential to the function of the domain, are encoded by gene segments split by an intron (type A1). If an intron mediated the duplication of a complete domain (type A2), the portion of the gene encoding the junction of the duplicated domains should contain the intervening sequence. Such A2 introns probably mediated the assembly of the three domains of ovomucoid⁸, the four domains of the immunoglobulin heavy-chain constant region⁹, the numerous 18-amino acid domains of collagen¹⁰, and the three domains of α -fetoprotein¹¹. If an intron participated in the novel association of two different functional domains (type A3), one would expect to find the same concordance of domain border and intron placement as seen in the A2 case. The A3 intron is probably responsible for the joining of a very similar NAD-binding domain to the different catalytic domains of the various dehydrogenases. Finally, if an intron arose as a divisive element (type B), it would separate a previously contiguous exon into two halves and would closely mimic the A1 case. However, one could still differentiate between the two cases whenever the divisive event occurred in a previously duplicated domain, that is, an A1 intron would be duplicated when the gene segment

Table 1 Concordance of protein domain borders with exon borders

	Domain border (amino acid no.)	Exon border (amino acid no.)	Intron
Nucleotide-binding site 1	75-90	76-77	IV
Nucleotide-binding site 2			
Catalytic domain	148-149	144-146	VI
Helical element	315-316	309-311	XI

Domain borders were determined by X-ray crystallography⁴, and exon borders by sequencing the chicken GAPDH gene⁶.

Table 2 Quantitation of half-hydrogen bonds within and outside protein segments encoded by chicken GAPDH exons

Exon	In	Out	% Out
II	0	9	100
III	24	12	33
IV	24	8	25
V	8	25	65
VI	18	12	40
VII	26	10	27
VIII	24	16	40
IX	2	27	93
X	22	4	15
XI	14	11	44
XII	38	2	5

The above data were calculated from the main-chain hydrogen-bonding scheme of Moras *et al.*¹³.

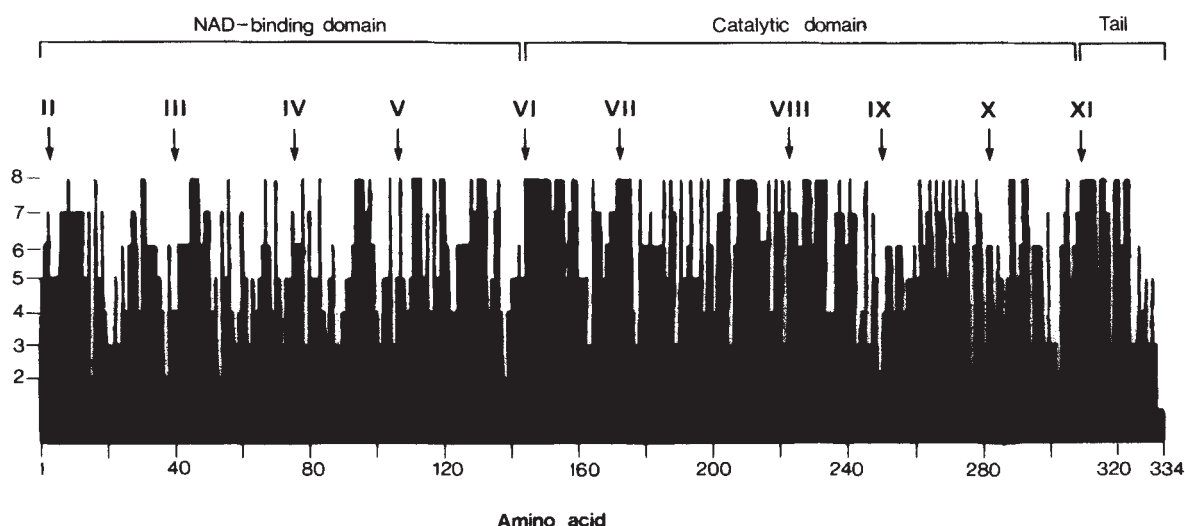


Fig. 1 Conservation of GAPDH amino acid sequences among seven species (eight genes). Amino acid sequences from *Thermus aquaticus*¹⁶, *Bacillus stearothermophilus*⁵, *Saccharomyces cerevisiae* (two genes)¹⁷, lobster¹⁵, pig¹⁸, chicken¹² and human¹⁹, were aligned and compared for sequence homology. Occasionally insertions or deletions of an amino acid were assumed to maintain the sequences in register, but in no case was the number of these assumptions per sequence greater than three. The amino acid residues of the composite GAPDH protein are given on the abscissa and the number of genes that agree at that residue plotted on the ordinate. Roman numerals indicate the positions of intervening sequences within the chicken GAPDH gene.

encoding the domain was duplicated, while a late-arriving B intron would have no homologue in the duplicated gene segment.

The chicken GAPDH gene lies within a 4.6-kilobase (kb) DNA fragment that was cloned and sequenced (Fig. 2). Comparison of this genomic DNA sequence with the previously published GAPDH mRNA sequence¹² revealed the existence of 11 introns; the amino acid codons split by these introns are given in Fig. 2. Intron I falls in the 5'-noncoding region of GAPDH mRNA and thus could not have participated in the evolutionary association of functional protein domains or their component subdomains, as suggested for all but one of the remaining introns. The locations of three of the intervening sequences correspond closely with the boundaries of four protein domains detected by X-ray crystallography^{4,14} (Table 1). Specifically, intron IV divides the two mononucleotide-binding domains (type A2); intron VI separates the catalytic domain from the dinucleotide-binding domain (type A3); and intron XI separates the helical element (shared by several different dehydrogenases) from the remainder of the protein (type A3). This concordance strongly suggests that introns participated in the construction of the GAPDH gene and therefore supports the hypothesis that RNA splicing occurred in very early organisms.

Examination of the hydrogen bonding of the peptides encoded by the various exons should indicate the degree of structural independence of each polypeptide from the rest of the protein.

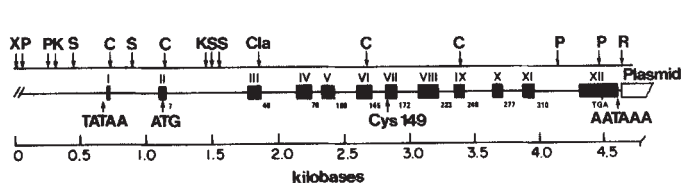


Fig. 2 Structural map of the chicken GAPDH gene. A detailed restriction map of the 4.65-kb *XhoI/EcoRI* fragment was obtained from a plasmid subclone (pGAD-13) of chicken genomic DNA, and the entire GAPDH gene was sequenced as reported by Stone *et al.*⁶. Restriction sites: ClaI, *Clal*; K, *KpnI*; S, *SmaI*; and P, *PstI*. Solid bars indicate GAPDH mRNA sequences; solid lines represent introns and flanking DNA. Small numbers indicate the terminal codon of each exon. The polymerase entry site (TATAA), initiation codon (ATG), catalytic cysteine (Cys 149) and poly-(A) addition signal (AATAAA) are also indicated.

The degree of autonomy of a protein segment may reflect the likelihood of it retaining its function if transferred into a new host protein by a gene fusion event. Table 2 gives an analysis of GAPDH hydrogen bonding, determined by X-ray crystallography¹³ (summarized in Fig. 3). The domains given in Table 1 share $\leq 15\%$ of their hydrogen bonds with residues of other domains. The polypeptide encoded by exon X is as independent by this criterion as the second mononucleotide-binding domain encoded by exons V and VI. Thus, we suggest that the introns flanking exon X are of the A3 type.

The exons separated by introns II, III, V, VII and VIII are highly dependent on others for the structure of the peptides they encode, and thus encode subdomains. Therefore, these introns must be of the A1 or B type. Introns VII and VIII separate three exons that each contain at least one essential component of the catalytic site. Exon VII contains the catalytic cysteine 149, exon VIII the destabilizing histidine 176 as well as the phosphate-binding lysine 191, and exon IX the other phosphate-binding residue, arginine 231 (ref. 14). Thus, introns VII and VIII probably participated in the initial A1 type assembly of the catalytic domain. The NAD-binding domain was formed by the duplication of a mononucleotide-binding unit⁴ and an A1 type introns that existed in the mononucleotide unit must also have been duplicated. Projected onto the protein, introns II and V exist in structurally equivalent sites in the NAD-binding domain (they each fall between the two β -sheets

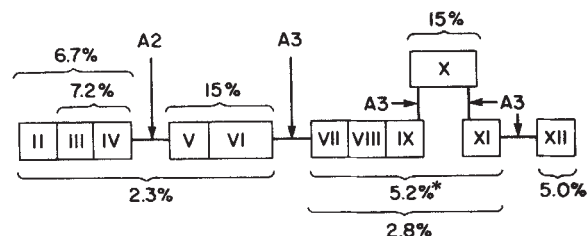


Fig. 3 Representation of hydrogen-bonding dependence of several regions of the GAPDH protein. Percentages were calculated from the main-chain hydrogen-bonding scheme of Moras *et al.*¹³. Roman numerals denote portions of the protein encoded by various exons of the chicken gene. A2 and A3 are intron types as described in the text. Calculation of % hydrogen bonding not including exon X is indicated by an asterisk. The number of an intron is the same as the exon that it follows.

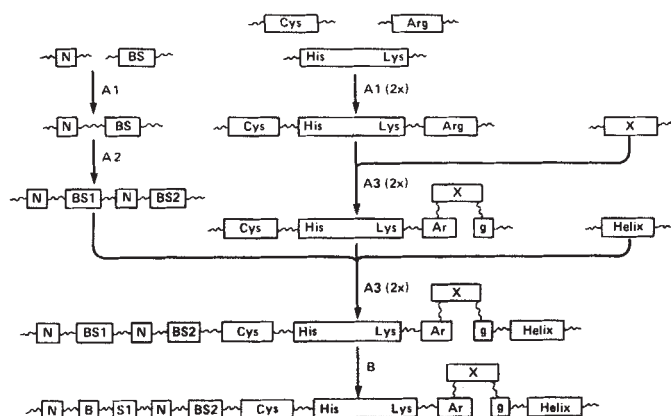


Fig. 4 Proposed ontogeny of the intervening sequences in the ancestral GAPDH gene. Functional domains and subdomains encoded by various exons of the GAPDH gene include the nucleotide-binding site (NBS), the polypeptide containing cysteine 149 (Cys), the polypeptide containing arginine 231 (Arg), the polypeptide containing histidine 176 and lysine 191 (His Lys), the polypeptide encoded by exon X (X), and the α -helical element shared by several dehydrogenases (Helix). A1, A2, A3 and B indicate the class of exonic connection mediated by the various introns, as defined in the text.

of a mononucleotide unit) and are thus most probably duplicated A1 introns. Conversely, intron III does not have a homologue in the second mononucleotide-binding unit and, therefore, probably arose by B-type DNA insertion into a previously intact exon.

Evolution of catalytic and regulatory sites in phosphorylases

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Glycogen phosphorylase (E.C.2.4.1.1) was the first enzyme shown to be regulated by allosteric effectors¹ and by protein phosphorylation². Transcriptional control of bacterial phosphorylases³ further extends the range of regulatory mechanisms by which phosphorylases contribute to the control of carbohydrate metabolism. Despite their regulatory differences, all known phosphorylases share catalytic and structural properties^{4,5} and a strongly conserved pyridoxal-5'-phosphate binding site⁶⁻⁹; this makes phosphorylases highly attractive for investigations into the evolution of regulatory mechanisms¹⁰. The primary⁶ and tertiary^{4,11-17} structure of rabbit muscle phosphorylase has been determined completely. Recently, comparable amino acid sequences from plants^{18,19} and bacteria²⁰ have been resolved. Here we report the sequence of 687 amino acids of *Escherichia coli* maltodextrin phosphorylase, deduced from a cloned *malP* gene sequence. Alignment of animal and bacterial phosphorylase sequences shows strong homology (48%) throughout 91% of the polypeptide chain enclosing the extrinsic catalytic region. Within this region, structural homology identifies a presumed phosphate-binding site from which the allosteric 5' AMP binding site of rabbit muscle phosphorylase might have developed. From the decreased alignment at the N-terminus and the presence of additional residues compared with bacterial phosphorylases, we conclude that the regulatory sequences that also carry the phosphorylation site in the muscle enzyme were joined to a presumed ancestral precursor gene by gene fusion after separation of the eukaryotic and prokaryotic lines of descent.

Figure 4 summarizes the hypothetical evolutionary steps in the construction of the GAPDH gene; this scheme predicts that when the genes of other dehydrogenases are sequenced, introns will be found dividing the mononucleotide-binding domains as well as the NAD-binding and catalytic domains. Similarly, it predicts that GAPDH gene sequences from other species will share the A-type introns but may differ in the number and position of the B-type introns. Sequencing of the genes for other glycolytic enzymes of higher eukaryotes should provide additional information about the evolution of primordial genes.

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We have determined the almost complete DNA sequence of the *E. coli malP* gene encoding maltodextrin phosphorylase²¹ using cloned chromosomal DNA fragments²² (for experimental details, see Fig. 1 legend). The sequenced portion contains 2,065 base pairs (bp) coding for 687 amino acids of the mature protein (Figs 1, 2). The N-terminal amino acid sequence of maltodextrin phosphorylase²³ and the DNA sequence encoding it²⁴ have been reported and were used to confirm the reading frame. In addition, >20% of the sequence was confirmed either by sequence analysis or from the amino acid compositions of tryptic peptides. The resulting 687 contiguous amino acids have a calculated relative molecular mass (M_r) of 78,000, representing 96% of the M_r 81,000 determined experimentally for the maltodextrin phosphorylase monomer⁹.

Comparison of the maltodextrin phosphorylase amino acid sequence (residues 1-687) with that of glycogen phosphorylase reveals an extraordinarily high level of homology. The best alignment requires the introduction of a few small deletions/insertions (Fig. 2) and the N-terminus of the mammalian enzyme appears to be significantly extended compared with the bacterial enzyme. The alignment results in 45% amino acid identity and 17% conservative replacements, giving 62% similarity overall in the sequenced part of maltodextrin phosphorylase. The homology becomes even more pronounced (48%) over residues 59-687. This high level of identity equals or surpasses the values obtained for similar comparisons with other key enzymes of carbohydrate metabolism²⁵.

In the catalytic region, all residues presumably involved in cofactor or substrate binding^{4,11-17} (see Fig. 2) are conserved in the primary structure, except position Glu 645. Note that the residues favoured for substrate contacts at the 'glycogen storage site' (positions 383-440 of rabbit muscle phosphorylase)^{26,27} are not equally well conserved, attesting to a more indirect 'allosteric' function of this site²⁸. However, ionizable and polar residues, considered to be important for the structure around the substrate-binding site¹⁵, are identical in both phos-

ATG TCA CAA CCT ATT TTT AAC GAT AAG CAA TTT CAG GAA GCG CTT TCA GGT CAG TGG CAG GGT TAT GCG TTA ATT TCT GCG GCT GAA ATG 90
 S Q P I F N D K Q F O E A L S B Q M Q R Y G L N S A A E M
 ACT GAT GCG CAG TGG TGT CTA GCA GTG AGT GAA GCA CTG GCG AAA ATG CCG GGT CAG CCA TTC GCG AAG CCG GTG GCG AAT CAG CCA 180
 P R O W M L A Y S E A L A E M L R A Q P F A K P V A N Q R
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 H V N Y I S M E F L I G R L T G N N L L N L G M Y Q D V Q D
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 S L K A Y D I N L T D L L E E I D P A L G N G G L R A
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 GGG ATT GCG GAT AAA GCG AAC GAA GCG GCG TGG GAG CCG GAT TTT ACC ATT ACC GAT CAA GCG TGG GAT CTT CCG GTT GTC AGC TAT 630
 G I G G K V T K D G R W E P E F T I T G Q A M D L P V Y G Y
 GGT AAT GCG GTC GCG CCG GTC GGT TGG TGG CCG ACG CAG CAT CCG TTT GAT CAG ACT AAT TTT AAC GCG GAT GAT GAT TTT 720
 R N G V A Q P L R L M Q I A T H A P F D L T V E K T M P G D
 CBT GCG CAG GCG AAT ACT GCG GAA AAA CTA GCG ATT CTT CTT CTA CCA AAC CAG CAT ACT GCG GGT AAA AAG CCG CCG 810
 R A E Q Q G I N A E K L T K V L Y P H D M T A G K K L R
 ATG CAG CAA TAC TTC CAG TGT GCG TGT TGG GTA GCG GAT ATT TTT GCG CCG CAT CTT CCG GCG GCG GAT CTA CCG GAA CTA GCG GAT 900
 M Q Q V F Q C A C S V A D I L R N H L A B R E L H E L A D
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 Y E V I Q L N D T H P T I A I P E L L R V L I D E H Q N S M
 GAT GAT GCG TGG GCG AAT ACT CCG AAG AAT TTT GCG ATT CTA GCG AAT CTA GCG AAT CTA GCG AAT CTA GCG AAT CTA GCG 1080
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 CCG CAG CTA GCG AAT CCG GAT TTT GCG GAT TAC AAG AAT GAT GAT GCG AAT CTA CTA CTA CTA CTA CTA CTA CTA 1710
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 GCG AAT GTC GCG AAG GAT GCG AAT GTC GGT GAA GAT AAT TTT GGT CAG AAG GTC AAG CAA GAT GCG AAG GAT AAT GCG 2064
 A H V E I A E K V G E A E I F G H Y T K D V K A I D

Fig. 1 The partial nucleotide sequence and derived amino acid sequence of the *E. coli* *malP* gene. A sequence of 2,065 nucleotides of the noncoding strand is shown. Nucleotides are numbered at the right-hand side of the figure, beginning with the first residue in the coding region. The sequence 1-163 and the upstream control sites (not shown) were determined previously by Débarbouillé *et al.*²⁴. The amino acid sequences (one-letter code) are numbered beginning with the first N-terminal residue (S) of native maltodextrin phosphorylase²³ following ATG.

Methods. Nucleotide sequence determination was carried out by the Maxam-Gilbert method³⁵ on 5'- and 3'-end labelled DNA fragments. DNA segments carrying the *malP* gene were recovered from two restriction fragments of the transducing phage Φ 80dmlA2 (ref. 21): a 5,800-bp *EcoRI*-*HindIII* fragment²² and a 2,400-bp *BglII*-*BglIII* fragment, overlapping with the *HindIII* site of the former (G. Weidinger, R.G. and D.P., unpublished). Approximately 93% of the nucleotide sequences was determined in both directions or by multiple sequencing. Underlined amino acid sequences were confirmed by peptide sequencing^{9,23} except for asparagine at position 649.

SRPLSQEKKRKQISVRLGAVENVTELKKDFDRHLHFTLVKNRNVATPRD 50
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 YNGVJALHSDLVKIDLFPEYHQLMPPNFHNTNGITPRRMLKCNPALAA 466
 IIAERIGEYISLDQLRLLSYVDDEAFIRDYAKVQKQENLKFAAYLER 550
 LLOKSQKEMANDLOQLIMLVKLADDAKFRDLRYVIRKANKVRLAEFYKV 516
 EYKVINPNSLFDVQVKRIHEYKRLNCLHVTITNRRKKEPNKFVYPR 600
 RTGIDINHOATFDITIKRLNEYKRLMLRLILALYKEITRQMPQADRYPR 566
 TVNIQGKAAPGYHMAKMIKLTITAGVYVNDHPYVGDRLRIFILEMTYVS 650
 VFLFGKAAPGYVLAKEIIFAIINKVADYVINDPLVGDRLKLVVFLPDYCVS 616
 LAEKVIPAADLSEQISTAGASGTGNMKMLNGLTITGTDGANVEMAE 700
 AEKLIIPAADLSEQISTAGASGTGNMKMLNGLTITGTDGANVEMAE 666
 EAGEENFIFGHRMEDVDRLDQRGNAQEYDRIPELRQIEQLSSGFFS 750
 KVGEENFIFGHTYKQVKAID-- 687
 PKQPDLFKDINMLMHDRKFVADYEYVCKQERVSALYKNPREWTRNY 800
 IRNIATSGKSSDRITIAQYAREINGVEPSRQLRPAPDEKIP 841

Fig. 2 Comparison of the primary structure (one-letter amino acid code) of glycogen phosphorylase from rabbit muscle (upper line) with the amino acid sequence of maltodextrin phosphorylase of *E. coli* (lower line). The amino acid sequences were compared using an algorithm based on the frequencies of substitutions in homologous proteins³⁶. Gaps (-) have been inserted to maximize similarity. Identical amino acid residues in homologous positions are boxed. Amino acid residues predicted to be involved in catalytic or regulatory interactions of glycogen phosphorylase are marked as follows: solid bars, residues involved in cofactor binding, substrate binding or catalysis; hatched bars, AMP effector binding site; ●●●, covalent phosphorylation site in phosphorylase a.

phorylases; this is in agreement with the assumption that evolution of proteins proceeds with conservation of three-dimensional structure^{29,30}. The overall shape of the protein remains unaffected even if one has to accommodate the deletions resulting from the comparison of the primary structure of both enzymes. Three of the deletions found in maltodextrin phosphorylase at positions corresponding to 209, 312 and 433 occur in loops of the muscle enzyme; the deletion at position 54 reduces the AA' helix¹² by one turn.

Glycogen phosphorylase is allosterically regulated by 5' AMP; the effector binding site is well characterized in the three-dimensional structure^{4,12,13,17}. Model building with the maltodextrin phosphorylase sequence reveals a conspicuous structural resemblance to a segment considered essential for binding of the phosphate moiety of 5' AMP in rabbit muscle phosphorylase. The positive charges of Arg 242 and Arg 308/309 of

rabbit muscle phosphorylase are involved in phosphate binding. At the corresponding positions of the *E. coli* enzyme, arginine residues seem to form a similar phosphate-binding pocket. The nucleoside portion of 5' AMP plays an equally important part in allosteric regulation of phosphorylases; all contacts with the nucleoside were detected within the N-terminal 80 amino acid residues of the rabbit muscle phosphorylase, but we found no structural homology in the bacterial enzyme within this region.

A second and more intricate control of glycogen metabolism in vertebrates is exerted by reversible covalent phosphorylation of phosphorylases. The modifying enzymes themselves are either under hormonal or nervous control³¹. However, until now, no covalent phosphorylation has been demonstrated for bacterial phosphorylases. In all cases investigated, a conserved phosphorylated peptide can be isolated from the N-terminus of the

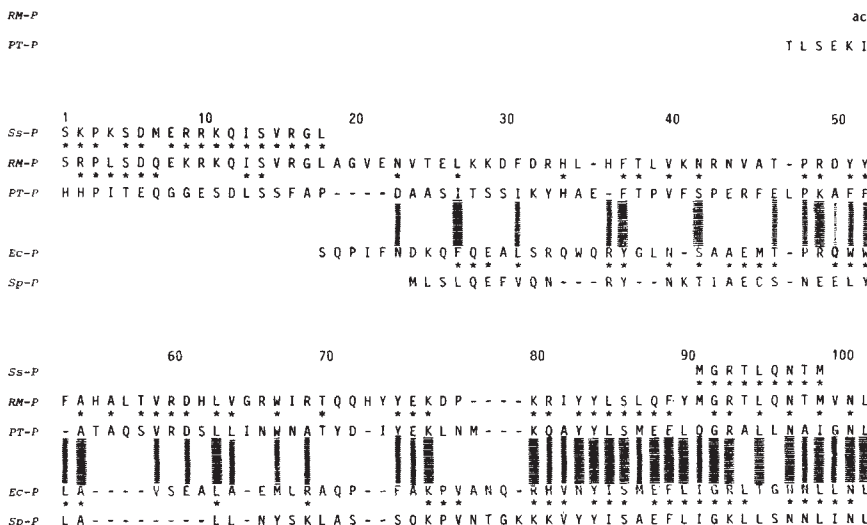


Fig. 3 Alignment of the N-terminal amino acid sequences of phosphorylases from eukaryotes: dogfish muscle (Ss-P), rabbit muscle (RM-P), potato tuber (PT-P); and prokaryotes: *E. coli* (Ec-P), *S. pneumoniae* (Sp-P). The best alignment³⁶ of all the sequences requires slightly different use of arbitrary gaps (-) compared with Fig. 2. Identities or favoured amino acid substitutions among two residues of either eukaryotic or prokaryotic phosphorylases are marked by *; identities or favoured substitutions among three to five residues of both lines are marked by vertical bars, roughly proportional in size to the alignment score³⁶.

vertebrate enzymes^{6,7,23}. From the alignment shown in Fig. 2 it is obvious that owing to the shorter chain length, the substrate site for phosphorylase kinase is not present in the bacterial enzyme. A more detailed inspection of the respective N-termini, including the corresponding amino acid sequences from dogfish⁷, potato¹⁸ and *Streptococcus pneumoniae*³², shows no sequences common to all five enzymes, but varying degrees of relatedness between individual pairs, reflecting their evolutionary distance from each other (Fig. 3). Only the N-termini of muscle phosphorylases are identical in size, related in composition and carry the regulatory phosphorylation site; the corresponding plant enzyme sequence is two amino acids longer, differing more in composition and cannot be phosphorylated. Conservation of sequences common to all five enzymes starts with residue 80 of rabbit muscle phosphorylase; an explanation for this derives from the arrangement of structural and catalytic elements that are well defined in rabbit muscle phosphorylase^{11,12}—residue 80 marks the beginning of the first β -strand of the central β -pleated sheet which forms part of the catalytic centre.

The nature of the presumed ancestral precursor gene is unknown, but we realize that catalysis in present-day phosphorylases does not depend on a unique composition of the N-terminus evolved independently of the catalytic core. Freedom from selective constraints in one part of the protein allowed the emergence of regulatory functions in vertebrates. The almost identical N-terminal sequences in vertebrates imply that the rate

of evolution became very slow after this control was established 450 Myr ago⁴.

There are several possible explanations for the onset of covalent regulation in phosphorylases. A model in which a preformed nucleotide sequence encoding a phosphorylatable peptide was fused to the ancestral phosphorylase gene is supported by the following evidence. A common peptide sequence distinguishes classes of protein kinase substrates³¹; the phosphorylatable sites in vertebrate phosphorylases conform completely to one of these prototype patterns. This points to a common origin of the corresponding coding sequences. Similar to the modular construction^{30,33} of phosphorylases proposed here, the cyclic AMP-binding domain of the bacterial catabolite gene activator protein and that of the mammalian cyclic AMP-dependent protein kinase constitute homologous modules in otherwise unrelated enzymes³⁰. Finally, we might ask how allosteric or covalent control evolved with time. From our data it is conceivable that the allosteric effector site developed from a primitive (secondary) phosphoryl-binding site³⁴. Completion of the allosteric ligand-binding site depended on the presence of the extended N-terminus (carrying also the phosphorylatable site) and profited from its genetic variability.

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Terrestrial catastrophism: Nemesis or galaxy?

CLUBE and Napier¹ have claimed recently that the hypothesis of an unseen solar companion triggering periodic mass extinctions^{2,3} can be eliminated. We disagree with their analysis. More importantly we point out that our theory has been completely misquoted. Their statement that "the binary system would not in general maintain the high eccentricity necessary for Oort cloud perturbations" attacks only one variant of the solar-companion theory, that given by Whitmire and Jackson², who conjectured that a high eccentricity was necessary to perturb the inner Oort cloud sufficiently to explain periodicity in mass extinctions. Our variant of the solar-companion theory³ does not in fact require an unusual eccentricity, e , any greater than the typical phase-space average value $e = 0.7$.

Two further points of Clube and Napier are clearly misleading. First, in stating that "among binaries with solar-type primaries, only ~1% have periods in excess of 0.3 Myr", they do not mention that this is caused by a purely observational bias, as wider pairs cannot be recognized by eye against the background stars on the sky. Instead, systematic searches for very wide binaries can be carried out only statistically, by performing a correlation test over an entire field to obtain binary candidates⁴, followed by a confirmation through, for example, radial velocity measurements⁵. Indeed, these studies^{4,5} have indicated a high incidence (~15% according to ref. 4) of very wide binaries with separations of ~0.1 pc (the expected original separation between the sun and the hypothetical companion star⁶, at the time of the formation of the solar system). Clube and Napier seem to have ignored this result of ref. 4, which is quoted in our paper³. Secondly, their statement that "only ~3% of binaries have eccentricities ≥ 0.75 " is again misleading as it does not apply at all to very wide binaries, for which the observations tell us nothing about the eccentricity^{4,5}.

There are other points on which we disagree. For example, we find a galactic modulation of comet perturbations to be significantly out-of-phase with periodicities in extinctions as well as cratering⁷; we estimate the expected lifetime of comets and wide binaries under the influence of passages with giant molecular clouds to be two or three orders of magnitude larger than Clube and Napier claim (P.H. and S. Tremaine, in preparation); we agree with P. Thaddeus and G. A. Chanan (unpublished) that galactic modulation of passages through interstellar clouds is orders of magnitude too weak to generate detectable periodicities in comet perturbations. These differences

between our respective theories will be resolved by more detailed research and we shall not address them here. What we do object to is the direct misquotation of our work, and the misleading statements which indirectly misrepresent our work. Indeed, the hypothesis of a solar companion star, generally referred to as Nemesis, remains as viable as when it was first proposed.

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CLUBE AND NAPIER REPLY—Davis, Hut and Muller are correct in stating that their version of the Nemesis hypothesis requires an orbital eccentricity $e \geq 0.7$ as opposed to $e \geq 0.85$ in the Whitmire-Jackson version, but the distinction is scarcely relevant. Stability, not eccentricity, is the real issue and our point¹ is that their contrived orbit (the major axis is assumed arbitrarily to be close to the plane) is unstable in a galactic environment dominated by molecular clouds. Furthermore, it has been emphasized² that, in arriving at the most probable theory for extraterrestrially-induced extinctions, it is necessary to consider all the relevant evidence; thus, it is not simply a question of abandoning the earlier "giant meteorite" scenario³ and arbitrarily embracing star-induced comet showers⁴ at ~26-Myr intervals⁵ brought on by a hypothetical unseen companion⁶. One must consider also the evidence for (1) a recently disturbed (~5 Myr) Oort cloud (inconsistent with the phase of Nemesis); (2) the well-known longer-term cycles⁷ in the terrestrial record (~30 and ~250 Myr being expectations of the galactic theory); and (3) the approximately constant time-averaged cratering rate over the last ~3,000 Myr (inconsistent with the declining flux implicit in the proposed evolution from an orbit with semi-major axis ~0.1 AU). Davis *et al.*⁶ (see also Muller *et al.*⁸) not only neglect the existence of

the molecular cloud system, but also clearly fail to address these points.

They also assert that the absence of very wide binaries is "caused by a purely observational bias". According to Retterer and King⁹, the absence of binaries with periods ≥ 0.3 Myr "represents a real absence of binaries rather than merely an inability to detect them. If wide binaries were present, Bahcall and Soneira [ref. 4 of Davis *et al.*⁶] would have been able to detect them in large numbers at separations up to 0.25 pc; instead they found no binaries wider than 0.1 pc". This is consistent with many earlier binary-star surveys, with ref. 5 in Davis *et al.*⁶) and with our statement¹ that "the proposed binary characteristics are very rare or absent amongst observed systems".

Finally, Davis *et al.* refer to unpublished work in support of the proposition that the galactic theory is untenable. It is of course not possible to respond to unspecified criticisms. What does seem clear is that, on present evidence, the Nemesis hypothesis is both contrived and unworkable.

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Activation of chromaffin cell Ca^{2+} channels by novel dihydropyridine

GARCÍA *et al.*, in their paper on the action of the calcium channel activator BAY-K-8644 on adrenal medulla cells¹, attempted to show that the radiolabelled calcium antagonist ³H-nitrendipine bound to membrane-fragment calcium channels. The data presented are, however, extremely contradictory. Thus, in the text it is reported that the dissociation constant (K_D) of ³H-nitrendipine is 1.18 ± 0.32 nM for 325.4 ± 136 fmol per mg of protein, implying that one homogeneous class of

sites was found. Figure 3 of ref. 1 shows the binding-inhibition curve of unlabelled nitrendipine, determined at the K_D concentration of ^3H -nitrendipine. The original data on specific binding (not defined by the authors) are presented in transformed form without giving either the total binding in d.p.m. (or c.p.m. or pmol) or the blank (nonspecific, unsaturable) binding. Accepting the data presented, we have calculated the 50% inhibitory concentration (IC_{50}) and the slope factor of this curve by computer analysis: the former is ~ 670 nM and the latter ~ 0.52 . In Fig. 3 legend¹ the IC_{50} is reported to be 1 nM, a difference of nearly three orders of magnitude. We considered the possibility of printing errors:

(1) In Fig. 3 of ref. 1 the scale runs from 10^{-9} to 10^{-4} M. If the scale was intended to extend from 10^{-12} to 10^{-7} M, the IC_{50} value in the legend would be correct. However, the slope factor of 0.5 would remain unchanged, which is inconsistent with a single site having a K_D of 1.18 nM. (2) If the legend should read: " IC_{50} values for nitrendipine and BAY-K-8644 were, respectively, 1 and 50 μM (instead of 1 and 50 nM) the figure legend would be consistent with the figure but not with the text, where nitrendipine bound with a K_D of 1.18 nM.

Alternatively, experimental errors could have been made, which could include the use of partially degraded, unlabelled 1,4-dihydropyridines (which are sensitive to light and oxidation) or dilution errors (see ref. 2). If the binding-inhibition curve for BAY-K-8644 is correct, then according to the printed scale, BAY-K-8644 has an IC_{50} of $\sim 30,000$ nM; this is the range in which the authors speculated (see Fig. 1 and text of ref. 1) that BAY-K-8644 acts as a Ca^{2+} -channel blocking agent. These potencies (assuming that the scales in the figures are correct) contradict those reported by Albus *et al.*³ in PC12 cells: in this study the 50% effective concentration (EC_{50}) of BAY-K-8644 in potentiating ^3H -noradrenaline release was 10 nM, close to the K_D (16.3 nM) of BAY-K-8644, determined by inhibition of saturable ^3H -nitrendipine binding in membrane fragments from these cells.

Note added in proof: Since the first description of calcium channel-linked 1,4-dihydropyridine receptor sites⁴, we have repeatedly emphasized⁵ the necessity of demonstrating the appropriate stereoselectivity of these receptors, regardless of the calcium channel subtype⁶. Other sites of lower affinity⁴, similar to those found by García *et al.*¹ in their displacement study, have the opposite stereoselectivity and have now been shown to be associated with the nucleoside transporter⁷.

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GARCÍA ET AL. REPLY—We appreciate the interest of Dr H. Glossmann in our letter¹ and thank him for his comments. Our paper was intended to show that the novel dihydropyridine BAY-K-8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) activates voltage-sensitive calcium channels of cat adrenomedullary chromaffin cells; numerous unpublished data and two more recent papers from our laboratory^{2,3} suggest strongly that this drug acts on such channels to cause their activation and/or increase their opening time, thereby enhancing Ca^{2+} entry with subsequent potentiation of catecholamine secretory responses, triggered by chromaffin-cell depolarizing stimuli.

The rationale in reaching this conclusion was based on three sets of experimental data: (1) BAY-K-8644 enhanced catecholamine release in response to weak, but not to intense, K^+ depolarizations; (2) the drug increased uptake of ^{45}Ca into isolated chromaffin cells at weak, but not at stronger, K^+ depolarizations; and (3) BAY-K-8644 displaced ^3H -nitrendipine bound to adrenomedullary membrane fragments.

Certainly, there is a printing error in Fig. 3 legend of our paper¹: the IC_{50} values for nitrendipine and BAY-K-8644 were 1 and 50 μM , respectively. These values are much higher than both the EC_{50} of nitrendipine (20 nM) required to inhibit catecholamine release from cultured bovine adrenal medulla cells⁴ and the EC_{50} of BAY-K-8644 required to potentiate K^+ -evoked catecholamine release from chromaffin and PC12 cells (10–50 nM)^{1,5}, to increase the peak inward Ca^{2+} -channel current in single guinea pig ventricular cells (50–100 nM)⁶ or to increase the K^+ -evoked contractions of rat vas deferens (1–10 nM; our unpublished results). Several factors can account for this discrepancy: (1) nitrendipine and BAY-K-8644 bind to different sites on the dihydropyridine receptor, as suggested by the non-competitive antagonism by both drugs of K^+ -evoked catecholamine release (Fig. 1b of ref. 1) and by the fact that both BAY-K-8644 and nitrendipine bind to high- and low-affinity sites on heart membranes^{7,8}. (2) The source of membranes used in our experiments was a crude microsomal fraction, containing large amounts of chromaffin granule membranes. In fact, a rough calculation of the

number of ^3H -nitrendipine binding sites (Ca^{2+} channels?) per μm^2 of surface area gave less than 1 channel per μm^2 , about 0.1 of the number found with patch-clamp studies on bovine adrenal chromaffin cells⁹. (3) Frozen or isolated membranes can contain modified Ca^{2+} channels or channel components having different properties; also, some endogenous substance that modulates the activation of chromaffin-cell Ca^{2+} channels may be lost during membrane isolation⁹. (4) Glossmann suggests that the photosensitive dihydropyridines used in our experiment may be inactivated; this is unlikely because we performed the experiment under a sodium lamp and both nitrendipine and BAY-K-8644 were pharmacologically active when tested on adrenal gland heart and vas deferens. (5) Tissue and species-specific variations in interactions of dihydropyridines with ^3H -nitrendipine binding sites have been described¹⁰.

Because novel dihydropyridine calcium channel activators are of great interest for neurosecretory research, we decided to publish our preliminary findings and included radioligand-binding displacement studies as they corroborated our conclusion that BAY-K-8644 was potentiating catecholamine release by acting on calcium channels; it seemed to us that the inconsistency between these data and the effects of the drug on secretion did not justify a delay in reporting our data.

We are presently performing radioligand-binding studies with several dihydropyridines using highly purified chromaffin cell plasma membrane preparations to define the molecular characteristics of such binding sites, their relation to voltage-sensitive Ca^{2+} channels and the number of such sites per cell.

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Taxonomy of death

Stephen Jay Gould

Extinctions. Edited by Matthew H. Nitecki.

University of Chicago Press: 1984. Pp.354. Hbk \$30, £27.50; pbk \$16, £14.75.

IN 1664, the great Jesuit scholar Athanasius Kircher published his *Mundus Subterraneus*, a massive treatise covering all objects found underground. The book is an aesthetic joy, but an intellectual puzzle to scientists of our time. It seems such a meaningless hodge-podge, a potpourri of unrelated objects — from dragons in caves, to fossils, to underground springs, to volcanoes — thrown together for the irrelevant reason that all lie beneath the Earth's surface. But Kircher's choice reflected neither stupidity nor expediency. Rather, his basic view of nature differed radically from ours, and taxonomies are the primary documents of changes in systems of explanation (I shall argue in a moment that the book here under review fails because its chosen taxonomy for death contravenes the probable causal structure of extinction as a biological phenomenon).

Kircher represents the last gasp of the pre-Newtonian non-mechanistic view of nature. He classified by common place and by similarity of form — conjunctions that had meaning in a world supposedly created at once to reflect God's transcendent good sense. A generation later, scientists had to classify by common genesis and common causal process — a criterion that made nonsense of an ordering by simple location underground.

In short, taxonomies are not neutral racks for the pristine facts of nature. They are theories that create and reflect the deep structure of science and human culture. A taxonomy is not just a ploy for convenient arrangement, but a hypothetical statement about the nature of things. This book, so admirable in nearly all parts taken separately, fails badly as an entity because its chosen taxonomy, seemingly obvious and "right" at first glance, is as superficial as Kircher's and for the same reason — it is based on a common appearance, not on common causes.

Extinctions is the latest of several volumes edited by Matthew H. Nitecki and based on the annual Spring Systematics Symposia at the Field Museum in Chicago. The book, loosely ordered by descending scale, includes articles on mass extinction (A. Knoll on its non-occurrence in plants, S. Stanley on temperature as a dominant cause), species extinction in geological time (A. Walker on the death of robust australopithecines, P. Martin on Pleistocene mammals), and extinction, local and permanent, in ecological time (J. Diamond on isolated populations, B. Patterson on mammals in mountain refugia and

T. Lovejoy *et al.* on Amazonian forests).

Death seems so obvious as a unifying theme, given its inevitability, and its great ineluctable common principle — you ain't there any more after it's over. But is death a unitary phenomenon? Is it a proper basis for a coherent taxonomy? In an honest prefatory essay, D.M. Raup writes:

In summary, the several papers in this volume present an almost frightening array of conceptual frameworks and interpretations of extinction. It is clear that there is little agreement on the basics.... Whereas there is always controversy in science, I submit that the range of views presented in this volume shows a level of disparity or downright confusion which is offscale.

I suggest that the reason for such maddening diversity is true disparity, not confusion. Maybe extinction is like being

underground — an improper joining of disparate objects grouped by a superficial similarity judged either irrelevant or even misleading by our best modern notion of causes. Maybe death in mass extinction is so different from death in the everyday struggle for existence that the simple fact of disappearance records no meaningful basis for common classification. But why have we so automatically considered extinction as a coherent "thing" across all scales of time and magnitude?

In its claim to encompass all scales, conventional evolutionary theory (the Darwinian modern synthesis) rests on a principle of reduction and extrapolation — natural selection is a struggle among organisms for reproductive success, and all phenomena of greater scale represent this process smoothly extended into larger and larger stretches of time. (Darwin's major statement about the geology of extinction, Chapter 10 in the *Origin of Species*, is one long argument for the continuationist vision — a claim that numbers and geographical range gradually dwindle before extinction and that the death of species is, therefore, a simple accumulation of individual losses in ordinary selection.)

IMAGE
UNAVAILABLE
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REASONS

Eighteenth-century impact theory — the frontispiece to Vol. I of Buffon's Histoire Naturelle, showing God hurling a comet at the Sun to form the planets.

Since death is so final and, when it comes, so quintessentially abrupt, we may note the irony that such gradualism has acted as a persistent bias to encompass all scales of extinction as extrapolation from ecological moments (with mass extinction as an even greater test of competitive mettle than natural selection in ordinary times). Raup continues:

Conventional dogma is being questioned and in some cases discarded. We are seeing a change from dominantly gradualist interpretations of natural phenomena to those that emphasize strongly chaotic events.

Obviously, if the Alvarez impact theory holds (and it now seems virtually established as a major factor in the Cretaceous debacle, with intriguing hints for its general validity as a primary trigger of disaster), then mass extinction is not competition by natural selection extended (by Darwin's metaphor of the wedge), but a separate process with rules for differential termination that cannot reduce to simple extensions or intensifications of ordinary adaptive struggles. Organisms cannot track or prepare for periods of cosmic disturbances (including major impacts) that occur once every 26 million years or so.

In his contribution, Paul Martin shows that extrapolation fails between coordinated deaths on small oceanic islands (inspired by human arrival) and extinctions of large mammals on several continents (where climate makes a stronger, perhaps dominant, contribution). Why, then, should we gather the vastly more different scales of this book together and regard death as fit for a common theory.

I offer no counsel of despair, but plead instead for hard thinking about proper taxonomies as guides to more adequate general theories. If we break up superficial categories such as death, or increasing diversity, in favour of events with common causes that respect the true differences across tiers of time and levels of hierarchy, then what shall we group together? Steno's great *Prodromus* superseded Kircher. The *Prodromus* is a treatise on taxonomy, though not usually so regarded. It makes a radical proposal — that the important objects of geology are solid objects within solids (from fossils and crystals in rocks to sediments in basins of deposition), and that the comprehensive category of solids within solids can be broken into sub-categories reflecting common causes (fossils that solidified before entombing rock vs crystals precipitated into spaces of a matrix already solid, for example). Such a taxonomy must have startled people used to ordering objects by Kircher's criteria of form and place — but it provoked the revolution that began modern geology. What is a proper taxonomy for the stuff of evolution and life's history? □

Stephen Jay Gould is Professor of Geology at the Museum of Comparative Zoology, Harvard University.

Magnify the soil

J.A. McKeague

Micromorphology of Soils.

By E.A. FitzPatrick.

Chapman & Hall/Methuen: 1984.
Pp. 433. £32.50, \$59.95.

THE subject of soil micromorphology, involving the use of microscopes in the study of soil, developed gradually after the publication in 1938 of W.L. Kubiena's pioneering book, *Micropedology*. Nowadays hundreds of soil scientists throughout the world examine soil thin sections by microscope to complement information obtained by other means, but only a dozen or two of them are specialists in micromorphology.

Dr FitzPatrick is one of those few experts and his individualism and enthusiasm are evident in this book, an attempt at a comprehensive treatment of the study of soil thin sections. Detailed instructions are given on the preparation of thin sections; apparently they work, as the numerous photomicrographs of soil features are of good quality. It is doubtful, however, that the details of equipment will be generally useful as equipment differs so much from one laboratory to another. Explanation of the use of the polarizing microscope for studying soil thin sections is excellent for readers without formal training in microscopy, while the chapters on properties of minerals and soil features in thin section

are clear and will be helpful to the intended users (students and soil surveyors). By contrast, the material on ancillary techniques, photography and teaching micromorphology seems too brief to be effective. Potential applications of soil micromorphology to fields such as agriculture and engineering are presented with some zest.

Difficulties over terminology, ever-present through the brief history of micromorphology, will harass readers of this book. Although Dr FitzPatrick declares in the preface his intention to use simple nomenclature and standard terms, he assails the readers with a bewildering chapter in which he recycles his own unique terminology for soil horizons and proposes some new, poorly-defined terms — resolution of nomenclature should be left to an international committee. Fortunately, Dr FitzPatrick complies with his intention of using standard terms in the "detailed description of thin sections", one of several appendices; although not meeting the "detailed" aspect, the descriptions are a model of the effective use of common words.

This first attempt at an all-embracing treatment of thin section studies of soil will be a useful reference book, especially for students. It is, however, an individualistic treatment rather than a state-of-the-art account of the subject. □

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The nuts and bolts of bosons

J.H. Mulvey

The Particle Connection: The Discovery of the Missing Links of Nuclear Physics.

By Christine Sutton.

Hutchinson/Simon & Schuster: 1984.
Pp. 175. £8.95, \$16.95.

NOTHING quite like it has happened in experimental physics since 1888, when Hertz detected the electromagnetic radiation predicted by Maxwell — an observation which set the seal on the unification of electricity and magnetism. Nearly a hundred years later, in 1983 at CERN, it was the turn of the W and Z bosons, the radiation of the "weak force" responsible for nuclear radioactivity and for the first step in the chain of events by which the Sun derives its energy. The parallel is close, for just as Maxwell joined electricity and magnetism the observation of the W and Z at CERN was a crucial confirmation of a theory which links electromagnetism with the weak nuclear force.

In the Deutsches Museum, Munich, the

apparatus used by Hertz sits on a shelf. Christine Sutton describes how the search for the W and Z required the use of CERN's particle accelerator, built by John Adams in a tunnel 7km in circumference, and a 2,000 ton particle detector housed in a huge cave 50m underground. Her book tells the tale of this great enterprise, an experimental *tour de force* which earned the two principal characters, Carlo Rubbia and Simon van der Meer, the 1984 Nobel Prize for physics. It is a sober, nuts and bolts account of the project and of the steps that led up to it. The preparation of the ground is thorough, perhaps to a fault, and we do not arrive at the W and Z experiment until Chapter 8, about two-thirds of the way through.

As a popular book on a major discovery the presentation would have benefitted from more explanation, more illustrations and less detail. But readers already hooked on the rapidly developing story of progress in understanding nature's forces will be well rewarded, especially by the very complete description of the pains taken by the physicists to be sure they had found real, not bogus, bosons. □

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Why zoos?

Gerald Durrell

Zoo 2000: A Look Beyond the Bars.

By Jeremy Cherfas.

BBC Publications: 1984. Pp.244. £12.95.

HAVING suffered from zoomania all my life, and having created a zoological collection of my own, I am nevertheless the sternest critic of zoos. They are exceptionally important institutions and will become more so in the future, but not all zoos are without blemish and many of them are appalling. It is however perhaps unfortunate that just at the point when good collections are in the transition stage, changing from Victorian menageries to modern establishments with all the accompanying technology, zoos as a whole are under attack from a vociferous group of so-called animal lovers, ill-informed for the most part and ignorant of the real function and value of a well-run zoological collection. It is therefore particularly pleasant to see the appearance of a book like *Zoo 2000*, which addresses and demolishes most of the criticism.

The zoo critics seem to be unaware of the fact that all responsible collections have been trying to set their houses in order for some time, since criticism (in too many cases well-justified) reflects on good and bad institutions alike. With a series of checks and inspections, the Federation of British Zoos, for example, has been striving to make bad zoos better and good zoos better still. This was voluntary and put into operation by the zoos themselves. Now, at long last, we also have legislation for the control of zoos, for it is quite astonishing that until recently anyone with sufficient funds could (with planning permission) start a zoo. One such "zoo director" once telephoned me to ask me how big a puma was. When, slightly startled at this ignorance, I enquired why he wanted the information, he told me that he had a cage six feet long by four feet wide and he wanted to know whether a puma would "fit it".

In his book, Dr Cherfas deals with this vexed problem of caging. From the anthropomorphic view, bars represent a prison, but to a highly arboreal animal, such as an orang utan or a gibbon, they are merely a form of forest canopy on which to swing and exercise. Regrettably too, a lot of people imagine that because an animal has an enormous area it must be happier than in a confined space, not realizing that in nature animals are confined or confine

themselves to a territory; nor is it generally appreciated that an animal can be just as unhappy in the sylvan glades of a safari park if it is looked after by unskilled people. In the book Cherfas points out very lucidly that game parks are nothing more than gigantic safari parks, but on the whole better run. It is useless for the anti-zoo body to keep prating on about animals being happier in the wild, when — minute by minute — the wild is shrinking and what little is left in terms of game reserves has to be managed in the same way that a large zoo has to be managed.

No one is suggesting that zoos can solve the problem of extinction by captive breeding, but they can certainly be of enormous help. The European Bison, the Père David Deer, the Hawaiian Goose and, in the case of my own organization, the Pink Pigeon, would not be in existence were it not for captive breeding. But it is nevertheless impossible, with all the goodwill in the world, to save all the species that are liable to become extinct in the next 50 or so years. I was, not many years ago, at an exceedingly depressing conference in San Diego, when I suddenly realized that we were all sitting round the table discussing which species we could save and which

species we would be forced, through lack of space or for financial considerations, to allow to become extinct. Still, the good zoos, of which there are many, can be a vital conservation tool and it is a step forward for all concerned that conservationists have now learned to value their usefulness in this respect.

Of course, zoos should cease to be as parochial as many of them are and be outward-looking. They should assist in the conservation of animals in the field and many of the more progressive zoos are doing this, such as the New York Zoological Society, Frankfurt Zoo and ourselves in Jersey. By such means, zoos will have been transformed from mere consumers of wild creatures to being their champions and helpers.

All this is made very clear in Cherfas's excellent book. It is the sort of account that I would like to press into the hands of any of those people who are anti-zoo, since it states so clearly and positively what some zoos are, what all zoos could be and how crucial are the functions they perform. □

Gerald Durrell is Founder and Honorary Director of the Jersey Zoological Park and the Jersey Wildlife Preservation Trust.

Stellar instability

D. Lynden-Bell

Physics of Gravitating Systems, Vols I and II.

By A. Fridman and V. Polyachenko.
Springer-Verlag: 1984. Vol. I pp. 468, DM 248, \$92.50. Vol. II pp. 358, DM 218, \$81.40.

THIS two-volume work is devoted to the dynamics of star systems in which each star moves in the smooth mean field of all the others. Galaxies and large star clusters are primary applications, but the authors concentrate on idealized models to illustrate the different instabilities which may occur. Thus the books largely deal with cooperative instabilities in stellar dynamics, which play a role analogous to the instabilities found in plasma physics.

After an exploration of the analogies between these fields, Vol. I contains calculations made with the small perturbations linearized about the equilibrium, while the second volume considers non-linear phenomena. The equilibria simple enough for detailed stability analysis are homogeneous slabs, thin sheets and spherical systems, and the stability of such systems is thoroughly discussed. The authors rightly emphasize the seminal contributions of Antonov to the theory of the stability of spherical clusters, and go on to cover generalizations and applications of his methods to particular models. Here, it is surprising that the general proof of the stability of

systems with isotropic velocity distributions which decrease monotonically is neither included nor mentioned.

The books include much of what is known about encounterless stellar dynamics, Fridman and Polyachenko demonstrating a wide knowledge of both Russian and Western literature prior to 1978. It is a pleasure to see this material organized and swept together between hard covers. Part of the reason for the length is that the authors prefer to give mathematical details for some rather specialized problems — for example the stability of models of spherical star clusters in which all the stars move in circles. One can argue that the truth cannot be understood without spelling out the mathematical steps, but it is not always easy for the reader to find out the general principles from among all the details. The theory of spiral waves in galaxies is treated but the swing amplification of waves is only touched upon. Presumably this is because Toomre's advocacy of it as the basic wave amplifier came too late to influence the books' contents. Some references from more recent literature (1982) are cited and discussed.

These two volumes are a guide through the literature rather than a pedagogical lecture course. They will be useful to research students who wish to know what has been done in this area, and contain the fullest treatment in the literature of the different instabilities. □

D. Lynden-Bell is Professor of Astrophysics and Director of the Institute of Astronomy, University of Cambridge.

● *The Pollen Loads of the Honey Bee: A Guide to their Identification by Colour and Form*, Dorothy Hodges' classic work of natural history first published in 1952, has been re-issued by the International Bee Research Association. The book is available through bookshops or direct from IBRA, Hill House, Chalfont St Peter, Bucks SL9 0NR, UK. Price is £26.50, \$42.50.

Astronomy from the darkroom

David W. Hughes

Colours of the Stars.

By David Malin and Paul Murdin.
Cambridge University Press: 1984.
Pp.198. £13.95, \$27.95.

Secrets of the Sun.

By Ronald Giovanelli.
Cambridge University Press: 1984.
Pp.116. £11.95, \$19.95.

WE HAVE been told that a picture is worth a thousand words, but how many more words is a colour image worth over black and white? Judging by the multitude of dramatic, beautiful and informative colour photographs in Malin and Murdin's book, the answer must be another thousand at least.

Colours of the Stars is much more than a collection of pictures, however. We are treated to a detailed discussion of the physiology of colour vision and led to bemoan the fact that, at low light levels, the eye effectively switches off its colour system. But photography of the sky can restore the reality of the colourful Universe. Ever since 1883, when Andrew Ainslie Common presented to a Royal Astronomical Society meeting his photograph of the Orion Nebula, the power of photography as an astronomical recording medium has been recognized. Colour didn't arrive until 1959, when William C. Miller used "fast" colour film and exposures of around four hours at the prime focus of the world's largest telescope. The usefulness of being able to record both intensity and colour in one image was immediately apparent.

Malin and Murdin concentrate on two things. First is a clear, practical explanation of the darkroom wizardry that, by colour separation, unsharp masking, photographic amplification, image superimposition and integration printing, can drag the maximum detail out of a photographic plate. Second are the end results. Page follows page of spectacular images of stars, nebulae and galaxies alive with colour. We are not, however, just left to admire their beauty. Colour is the clue to a host of physical and chemical processes: nebulae reflect and emit; forbidden transitions abound; dust scatters both forwards and backwards; each element has its signature; temperature, pressure and velocity can be revealed.

There is much that is new in this book and everything is very clearly explained. The authors' enthusiasm for their subject is apparent throughout and their desire to share this enthusiasm and to help the reader to delve more deeply is furthered by the provision of an impressive reference list.

Moving from countless millions of stars submerged in swirling clouds of gas and

dust to one star a mere 150,000,000 km away brings us to the Sun. Instead of recording a point of starlight we can now reveal minute details of sunspots, granulations, fibrils, canopies, plages, spicules, coronal holes, prominences, flares and so on. The common ground between Giovanelli's book and *Colours of the Stars* is the reliance on photography. With the Sun, however, the emphasis is on narrow wavelength bands moving, for example, slowly up the limb of the Hydrogen Alpha line, thus enabling us to sample different layers of the photosphere, produce magnetograms to delve into the magnetic structure and use the Doppler shift to plot out the velocity variations in the convective regions.

The Sun is a very complicated object — the more so, it seems, the more we find out

about it — and Giovanelli points towards the five basic "solar problems": the cause of sunspots and their cycle, the structure of the convective zone, the variation of spin with latitude and depth, the mechanism responsible for coronal heating and the cause of flares. But while the book is billed as being intended for non-specialists and non-scientists, and mathematical equations and scientific jargon are taboo, it still does not make our Sun an easy object to understand. The author tries hard to explain what is going on but should have given himself more room. A picture might well be worth a thousand words, but without the picture the words need writing. □

David W. Hughes is Senior Lecturer in Astronomy and Physics in the Department of Physics, University of Sheffield.

Spark o' nature's fire

David R. Rosseinsky

Electron-Molecule Interactions and their Applications, Vols 1 and 2.

Edited by L.G. Christophorou.
Academic: 1984. Vol. 1 pp.699, \$80, £67.
Vol. 2 pp.678, \$85, £71.

WE FEW, who risked incredulity if not suspicion at our 1960s belief that the scientific event of the decade — dominated, remember, by twisted helices, parity, lasers and bizarreness — was the experimental establishment of the electron affinities of the halides, will, alas, find here but a one-line entry noting that result. But then we should have been warned, by both title and preface. If chemistry is the interesting part of physics (G.N. Lewis this, I think) then these tomes qualify as chemical, but in fact the authorship is largely North American physicist, accounting no doubt for the particular selection of references (200–450 per chapter).

The editor, a major contributor to studies in the field, has co-authored five of the thirteen articles, four in the second volume in which the stress is on applications of the preceding fundamental studies. He has aimed ultimately at emphasizing the role of slower electrons, which are thus more reactant in nature: more particle, less wave. Elastic scattering of electrons by molecules is dealt with as standard particulate scattering, where scattered intensity is represented as differential (scattering-angle dependent) cross-section, integrable to total scattering cross-section of target species, each dependent on incident energy; the now numerous theoretical analyses are clearly outlined. Reference is included to electron-molecule interactions in the atmosphere of the Earth (causing aurorae) and of other planets. Exciting, ionizing and dissociating collisions, transient electron attachment in

resonance interactions and a further variety of electron attachment and detachment processes are analysed in terms of the cross-sections, and inferred potential energy surfaces and molecular mechanisms. The events are complex — consider even the basic processes of electron capture, autoionization, dissociation or stabilization of a simple species AX^- — and the profundity of interpretation presented here represents an impressive scientific achievement.

Volume 2, emphasizing applications, starts with "Electron Transfer Reactions", nearly all in the gas phase. Replacing the physics-versus-chemistry jest by a more relevant contrast of small-molecule versus condensed-phase studies, the tenor of these volumes lies with the former — condensed-phase electron transfer is included but fleetingly. Studies are reported of electron/molecular-cation recombination (dissociative recombination), which also occurs in interstellar space and the upper atmosphere of the Earth. Electron transport in gases, giving an alternative source of scattering cross-section, relates to dielectric use. Electron interactions in liquids are briefly viewed in the light of known electron-gas interactions. Technological applications also related to the fundamental studies arise in laser usage, lamp construction, dielectric applications, and uses of plasmas. Extensions to biology — involving condensed systems — are tenuous. By contrast, the final comprehensive chapter on electron affinities, methods and results, making up a third of Vol. 2, would on its own serve as an invaluable monograph for physical scientists, and is quite admirable.

In general the prose is, let us say, not overburdened with elegance, and some sections have suffered quirky proof-reading. But these are trivial defects in an authoritative, almost encyclopaedic, opus. □

David R. Rosseinsky is Reader in Physical Chemistry at the University of Exeter.

Peptides and oligonucleotides

New products on the market include kits for manual and automated synthesis of oligonucleotides

● Applied Protein Technologies has added an instrument for the automated synthesis of peptides to its range. The instrument is modular and is microprocessor controlled with accurate reaction monitoring. Fifteen randomly accessible amino acid reservoirs are supplied and a wide range of reaction vessel sizes is available. The new synthesizer complements APT's existing line of services, which include custom peptide synthesis, peptide purification, and solid supports for peptide and protein immobilization.

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● A recent addition to LKB Instruments' list of application notes on peptide purification is entitled "Purification of Charged Isomers of Human Growth Hormone by Electrofocusing in Immobilized pH Gradients". The note (Application Note 325) details how the b- and c- components of human growth hormone and their sub-components can be recovered on a preparative scale on LKB Immobiline immobilized pH gradients.

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Quick-Blot for mRNA immobilization.

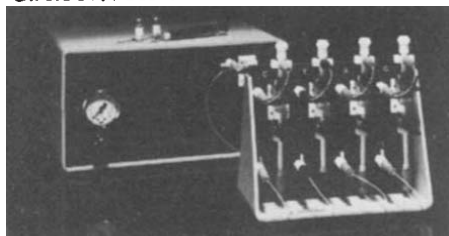
● Using the Schleicher & Schuell Quick-Blot kit, 120 mRNA or DNA samples can be tested. In conjunction with the Minifold I and II multifiltration units (devices for simultaneously filtering 96 or 72 samples) different mRNA samples can be immobilized from whole cells, bacteria or viruses on the cellulose nitrate within a few hours. The 0.32 or 0.06 cm² filter surface area in the Minifold I and II allows as much as 200 or 40 µg mRNA to be immobilized per sample. All samples remain biologically active because no baking is required. The Quick-Blot kit for selectively immobilizing mRNA can also be used for isolating DNA from whole cells and for the purification of mRNA for hybridization, reverse transcription or translation.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

● The Pharmacia range of pUC family plasmids has been extended by the introduction of plasmids pUC-18 and pUC-19. Both can be used as general purpose cloning plasmids, expression vectors or for DNA sequencing using the Pharmacia M13 DNA sequencing primers. The new plasmids are an improvement on the earlier members of the pUC family in that they contain higher restriction site densities in the multiple cloning sites. pUC-18 and pUC-19 have been shown to grow well and give a high copy number.

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Four simultaneous DNA separations can be performed with Cruachem's new module.

● New from Cruachem Ltd. is a four-column module which can be connected to a manual or semiautomated DNA synthesizer to offer two options. First, up to four simultaneous DNA syntheses can be run in parallel and second, multiple DNA syntheses can be performed following the 'segmented synthesis' technique. Using the latter technique 125 oligomers have been prepared simultaneously.

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● Bachem Inc. can now supply all the atrial natriuretic factors so far isolated. These can be supplied in milligram to gram quantities. Bachem can also produce milligram to gram quantities of custom synthesized peptides under GMP, with all the work done in the strictest confidence. Amongst the new products listed in the latest Bachem catalogue are a CRF antagonist, a somatostatin antagonist, the neuromedins and the neurokinins.

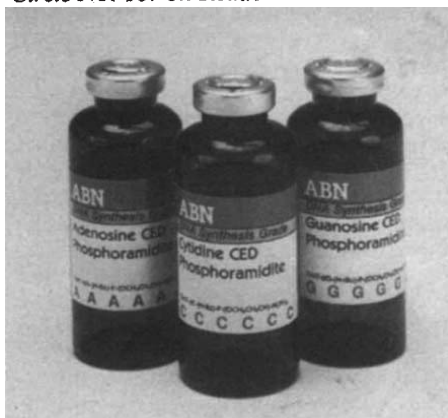
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● The Sepragel range of pre-cast polyacrylamide electrophoresis gels are pre-cast gradients available in the popular 16 × 12 cm gel sizes. These thin 1.5 mm gels provide good resolving power, high quality and reproducibility in autoradiography and Western blot techniques. Gels are supplied in two buffer systems (SDS and non-SDS) and four gradient ranges (3–27%; 10–20%; 3–12%; and 17–27%). For rapid evaluation, inexpensive Introductory Kits are available containing a detailed 16-page instruction manual.

Circle No. 106 on Reader Service Card.

● Du Pont has introduced a new ultra-stable high performance gel filtration column designed specifically for the separation of peptides and proteins. The Zorbax BioSeries GF250 allows fast, high efficiency separations of peptides and proteins in the 200 to 400,000 dalton range, with extreme stability and very high recoveries. The column is based on a patented zirconia-clad silica packing material, to which is bonded a hydrophilic stationary phase. The packing, of 4-µm particle size, is stabilized with zirconia to allow operation at the relatively high pH ranges required for successful peptide and protein analysis. The high efficiency (a minimum of 60,000 plates per metre) allows baseline resolution of, for example, IgG from bovine serum albumin as well as the light from the heavy chain of IgG. These analyses are generally completed within as little as 20 min. Typical recoveries are greater than 95% and the column can accept up to 250 µg of sample.

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One-step deprotection is a feature of American BioNuclear's CED phosphoramidites.

● CED phosphoramidites are a new generation of reagents for DNA synthesis available from American BioNuclear. Prepared with a B-cyanoethyl phosphate protecting group, oligonucleotides synthesized using CED phosphoramidites can be conveniently deprotected in one step using ammonium hydroxide. Thiophenol treatment at the completion of synthesis is completely eliminated. Each lot of CED phosphoramidites is functionally tested to guarantee coupling efficiencies of 97–99%. These CED phosphoramidites are packaged in 0.5 and 1.0 gram sizes in amber, septum vials to fit most automated DNA synthesizers; a special 'Quick-Pak' is also available pre-packaged to fit the Systec Model 1450A synthesizer.

Circle No. 108 on Reader Service Card.



The solid-phase peptide synthesis, the new Syn-Thor 2000.

● The Syn-Thor 2000 is the latest solid-phase peptide synthesizer from Peptides International of Louisville, Kentucky. The Syn-Thor 2000 package includes a two-disk Syn-Thor program as well as the IBM PC computer, equipped with additional memory and two 320 KB disk drives. Also included is an IBM colour monitor and all necessary interphase boards and cables. The synthesizer features a new delivery system with selective computer-controlled pressurization plus self-cleaning lines and separately programmable wash steps to eliminate cross contamination.

Circle No. 109 on Reader Service Card.

● Anglian Biotechnology now produces 64 restriction endonucleases. *AocI* is a recent addition. An isoschizomer of *MstII*, *AocI* recognizes the sequence CC/TNAGG and is useful for sickle cell anaemia diagnosis. This enzyme is stable; excellent ligation and recut characteristics are achieved. Other new restriction enzymes from Anglian are *Dra II* and *Dra III*, which recognize the sequences PuG/GNCCPy and CACNNN/GTG respectively.

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● New from Beckman Instruments, Inc. is a reconfigured albumin assay for use on ICS II and Auto ICS immunochemistry analysers. This reformulated test measures serum albumin up to 6,000 mg per dl. A new calibrator calibrates at a midpoint of the normal curve, 3,000 to 3,500 mg per dl. Midpoint calibration offers greater accuracy across the measuring range and improves low end precision where normal patient values tend to fall.

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● Peninsula Laboratories Europe Ltd has been set up in the United Kingdom to supply a wide range of synthetic hormones to European customers. A 24-hour by-return service is provided. In stock are such items as growth hormone releasing factor (human, rat, ovine and bovine) and the prohormone fragment, corticotropin releasing factor (human, rat, ovine) and its inhibitors and precursors, atrial peptides and dynorphins. Also, a complete range of peptide hormone antisera for RIA and immunocytochemistry is available. A free catalogue can be obtained via the reader service card.

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● 'Peptide and Protein Sequences' is one of six new topics added (from January) to the biweekly current awareness service, *Biosis/CAS selects*. The five other topics are: Cancer and Nutrition; Food and Drug Legislation; Genetic Manipulation in Plants; Leukotrienes and Slow-Reacting Substances; and Lymphokines. The series is a joint venture of Bio Sciences Information Service and Chemical Abstracts Service.

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● A calibration protein set for SDS gel electrophoresis covering the range 20,000–340,000 may be obtained from Boehringer Mannheim. The materials used in the kit are specially purified and may be used in the systems according to Laemmli, Shapiro and Weber & Osborn. Linear calibration curves over the whole range of 20,000 to 340,000 have been achieved in SDS gradient gels. Approximate linearity in partial ranges can be achieved using gels with a constant acrylamide concentration. The proteins contained in the kit are: α 2 macro-globulin, phosphorylase *b*, glutamate dehydrogenase, lactate dehydrogenase and trypsin inhibitor.

Circle No. 114 on Reader Service Card.

● DNA synthesis support columns for the Applied Biosystems Model 380A DNA synthesizers are now available from American BioNuclear. Manufactured from the highest quality materials, end caps and columns are precision machined from inert plastic to give leak-free performance. Retaining filters are constructed from Teflon mesh allowing maximum flow rate, without loss of support. Aluminium crimp seals are secured by hand and each column inspected before shipment. The column packing material is derivatized long-chain alkylamine controlled pore glass, (LCAA-CPG). Prepared to a loading of 20–30 μ mole per gram, LCAA-CPG achieves high coupling efficiency and good recovery of synthetic DNA. ABN support columns are available in 1.0 μ mole and 10 μ mole sizes.

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● The new aqueous cyano HPLC columns from IBM are claimed to decrease start-up time and increase the reproducibility of chromatography analysis. The columns are packed with a proprietary 5µm spherical silica stationary phase that yields exceptional selectivity and efficiency. The quality control of these columns includes the separation of PTH amino acids. With specifications of 55,000–80,000 plates per metre and an asymmetry range of 0.9 to 1.2, they are efficient enough to accomplish the most complex PTH amino acid separations. The 4.5mm internal diameter IBM aqueous cyano columns are offered in three standard column lengths: 50mm, 150mm and 250mm, allowing users to choose the correct efficiency while minimizing both the time of analysis and solvent consumption.

Circle No. 116 on Reader Service Card.

● The quality of terminal deoxynucleotidyl transferase produced by Pharmacia Molecular Biology Division of Milton Keynes has been further improved by the introduction of a new oligonucleotide digestion test for exonuclease activity. SDS-polyacrylamide gel electrophoresis indicates that Pharmacia TdT is over 95% homogenous. The new test is an addition to the standard purity checking for endonuclease and exonuclease activity.

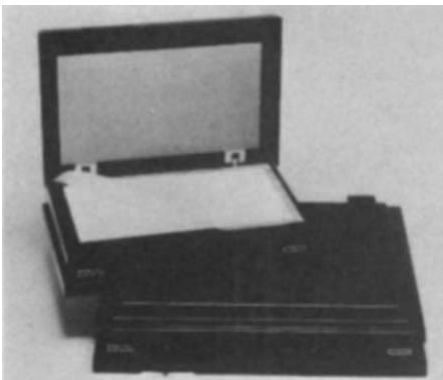
Circle No. 117 on Reader Service Card.

● A high quality reverse transcriptase is now available from Bio-Rad. The enzyme is subject to vigorous quality control to ensure its suitability in cDNA cloning applications. These tests include the determination of enzyme concentration; time-course synthesis; cDNA synthesis from ovalbumin mRNA; test for degradation of cDNA and test for RNase activities. As a result, batch-to-batch variation is minimized. Provided with the enzyme are ovalbumin mRNA, RNA size markers and protocols to allow the customer to reproduce Bio-Rad's quality assurance functional test as a positive control. Bio-Rad reverse transcriptase is available in 1,000-unit vials.

Circle No. 118 on Reader Service Card.

● Boehringer Mannheim have reintroduced reverse transcriptase to their programme of reagents for molecular biology. The new preparation shows a high volume activity (greater than 10,000 units per ml) and the units are nmolar units not pmolar units. The enzyme is guaranteed RNase and DNase free and fully tested for its performance in cDNA synthesis. Use the service card for more details of this enzyme together with other reagents for cDNA cloning.

Circle No. 119 on Reader Service Card.



The new slab-gel dryer from Biorad.

● Bio-Rad's slab gel dryers produce flat, dried gels with a smooth glossy surface, and provide excellent preservation of high resolution gels for SDS-PAGE, 2-DE, DNA sequencing, and IEF. The gel is dried by controlled heat, evenly distributed on the top surface of the gel, by a heater element suspended from the lid. The heater maintains the temperature at 80°C for rapid drying, or at 60°C for gels which have been impregnated with fluors for autoradiography. The vacuum is distributed evenly by a porous gel support. The liquid in the gel is pulled directly down (not at an angle). The heat from above combined with the evenly distributed vacuum dries the gel to a smooth flat sheen. Soft IEF gels dry with high resolution separations clearly preserved, because the evenly distributed vacuum prevents sideways stretching or distortion of the gel.

Circle No. 120 on Reader Service Card.

● LKB's new purification system, Ultrochrom GTi, offers multimode separation of macromolecules, authentic high performance and complete biocompatibility. Designed for the biochemist working with proteins, peptides, nucleic acids and even carbohydrates, GTi provides fast separation, high resolution and high biological activity (results range from 91% to 99%). The Ultrochrom GTi can use gel filtration (GFC), ion-exchange (IEC) or hydrophobic interaction chromatography (HIC), so the most appropriate chromatography can be exploited for any particular application. In addition, the total separation capability provided by the system enables it to be used for mainstream reversed phase chromatography, including microbore and fast LC. All components are fully compatible with LKB autointelligent HPLC instrumentation.

Circle No. 121 on Reader Service Card.

● Pierce Chemical Company of Rockford Illinois has introduced a complete kit for the manual synthesis of defined sequence oligodeoxynucleotides. Each kit contains all the reagents necessary to synthesize four 20-nucleotide oligomers. The Pierce manual DNA synthesis kit, or Nucleolink, uses phosphate triester chemistry based on the methods of Khorana and Itakura. The coupling reaction is performed in a pyridine solution containing mesitylenesulphonyl chloride as the condensing agent. The Nucleolink kit contains all essential reagents and accessories, including step-by-step instructions, to construct up to 10.4 units of a 15-nucleotide oligomer quickly and efficiently. A single coupling cycle takes only 15 minutes and the yield is greater than 95% per residue. The starting nucleoside is bound to an insoluble silica support with the 5' terminus protected with dimethoxytrityl (DMT). Following the removal of DMT, this starting nucleotide is condensed with another 5' DMT protected nucleotide which has a reactive 3' terminus. Synthesis progresses rapidly from the 3' to 5' end of the oligodeoxynucleotide.

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by5

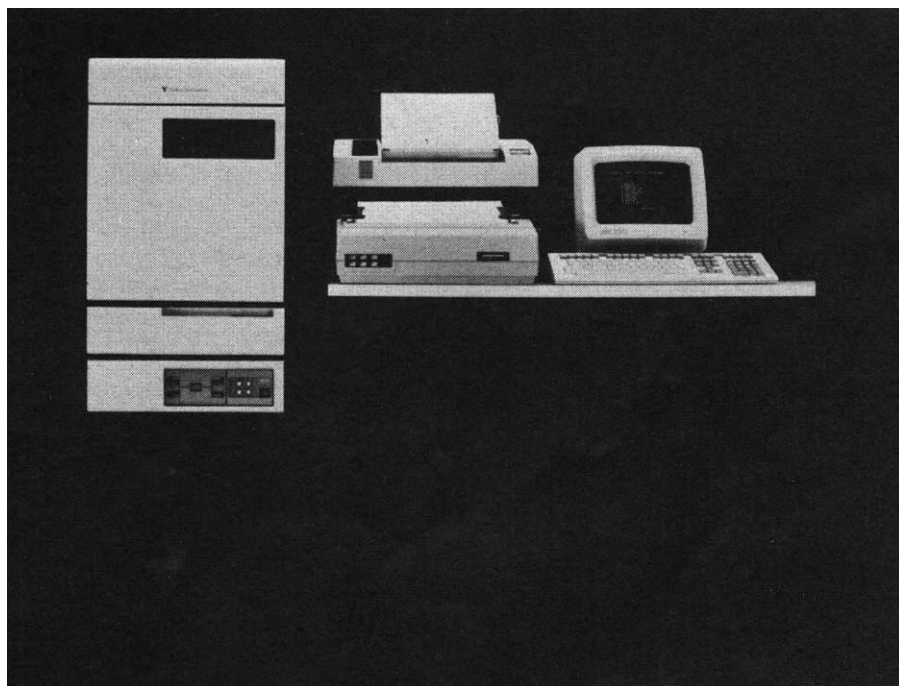


Image processing is a component in the Vickers Scanagel.

● Scanagel, new from Vickers Instruments, is an easy-to-use, stand-alone instrument designed to give the protein chemist fast reliable analysis and comparison of two-dimensional electrophoretic gels. Scanagel analyses wet gels or autoradiographs of any size from 4×4 cm to 20×20 cm. The sophisticated image processing software automatically carries out background corrections, interprets spot boundaries, resolves overlapping spots, measures spot location and intensity, and compares matched and unmatched peaks between gels. All programs are executed from the computer analysis menu, allowing easy system operation. Five results presentations are possible, including peak lists, two-dimension ellipse and contour plots, three-dimension surface plots and gel comparisons.

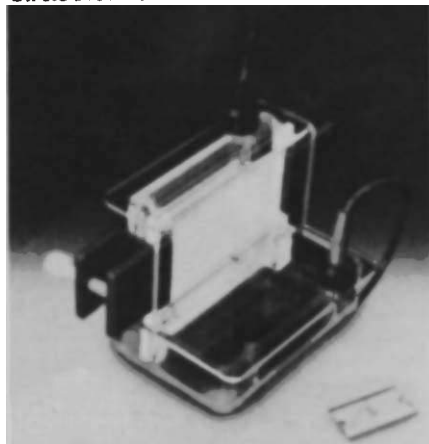
Circle No. 123 on Reader Service Card.

● High performance, rapid gel filtration is now possible with the two different types of new Superose columns from Pharmacia Biotechnology. The glass HR 10/30 (10×300 mm dimensions) columns, pre-packed with rigid $10 \mu\text{m}$ diameter, cross-linked agarose beads, provides a rapid, high resolution gel filtration system. The high stability of Superose to dissociating agents and extreme pH permits a free choice of eluents and allows scrubbing with sodium hydroxide. Scrubbed columns have a long lifetime and can be used repeatedly. Superose 12 is designed for separation of molecules with molecular weights between 1,000 and 300,000. High resolution separations of small tryptic peptides have also been obtained. Superose 6 is more porous and is appropriate for separation of large molecules such as nucleic acids (or restriction fragments), large proteins and aggregates.

Circle No. 124 on Reader Service Card.

● Chemical Dynamics Corporation has announced a new coupling reagent for oligonucleotide synthesis 1-(8-quinolinesulphonyl)tetrazole (QSTET). In a comparative study, QSTET gave a 92% yield of coupled product with no sulphonation by-product in a reaction time of only one hour.

Circle No. 125 on Reader Service Card.



Small-format electrophoresis from Hoefer.

● The Hoefer Mighty Small vertical electrophoresis unit offers fast results in a small format. The hand-sized unit is 11 cm wide and casts a gel 8.3×7.3 cm in 0.5, 0.75, and 1.5 mm thicknesses. The unit is designed to draw heat away from the gel and distribute it throughout the buffer by sealing the gel sandwich against the vertical upper buffer chamber so that the inside plate of the sandwich functions as a wall of the buffer chamber. The plate separating buffer and gel is made of aluminium oxide 30 times more heat conductive than glass. These features prevent gels from overheating. An accessory casting container permits the casting of 10 uniform concentration or gradient gels at a time.

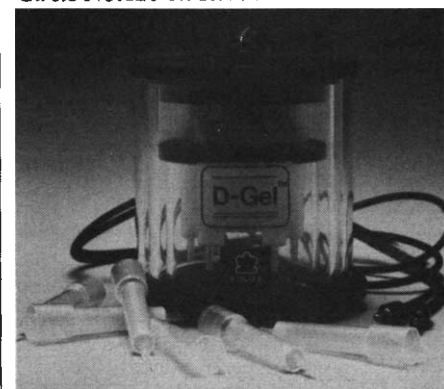
Circle No. 126 on Reader Service Card.

● The new Chrompack on-line, precolumn derivatization HPLC Autosampler makes complicated sample pretreatment redundant. It is particularly suitable for the analyses of primary amino acids using OPA methodology. The sample liquid and OPA reagent are mixed automatically and kept in a reaction coil during the reaction time; after which the mixture is injected into the separation system of the chromatograph. This procedure is claimed to result in analyses with much better reproducibility than achieved by elaborate manual sample pretreatment. The Autosampler is based on the Spark design, with a capacity of 125 samples.

Circle No. 127 on Reader Service Card.

● Oncor, Inc. last month released what are claimed to be the first commercially available probes for the unequivocal identification of clonal populations of B and T lymphocytes by hybridization analysis. The DNA probes detect B and T-cell gene rearrangements and offer specific advantages over antibody tests in the assignment of lymphoid cell type when antigenic markers are absent.

Circle No. 128 on Reader Service Card.



The Kontes device for DNA recovery.

● The Kontes D-Gel electrotransfer system is a rapid, inexpensive system for DNA recovery from electrophoresis gels. DNA restriction fragments, synthetic oligonucleotides, primers and linkers can be easily recovered from agarose or polyacrylamide gels. The basis of the D-Gel system is electroelution of nucleic acids from electrophoresis gels onto DEAE-cellulose or DEAE-anion exchange resin. Typical recovery of DNA fragments smaller than 10 kilobase pairs is 80-90%. Particularly effective when dealing with synthetic oligonucleotides, the D-Gel achieves a 90-100% recovery rate. In addition, the D-Gel obviates the phenol extraction required by the low melting agarose technique and substantially improves nucleic acid recovery.

Circle No. 129 on Reader Service Card.

● Porcine platelet derived growth factor is now available from Bioprocessing Ltd of Consett, UK. The material can be supplied in small or large quantities from reproducible batches, it is highly stable, biologically comparable to human PDGF.

Circle No. 130 on Reader Service Card.

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Technicians need new skills

from Richard Pearson

Two recent reports from the Institute of Manpower Studies indicate that the adoption of new technologies in the workplace introduces the need for, but benefits from, retraining of staff, particularly technicians.

A FAILURE to adopt new technology is seen as the one sure way for declining competitiveness in the world economy. The lesson applies equally well for companies and nations, while individuals who do not understand and adapt to the skill needs of the new technologies are also becoming increasingly disadvantaged in the labour market. While the shortage of professional level scientists and technologists has been the focus of many reports in recent months, skill constraints at technician level have been highlighted in the latest Government Skill Shortages report. It says that industry needs to recruit and train twice as many technicians as at present. Equally important is the need to consider the requirements of employees when introducing new technology. Even high-tech pharmaceutical companies are not immune to such difficulties. Thus a research laboratory with all the latest instrumentation for monitoring and analysing data from experiments recently had great difficulty in upgrading the skills of its maintenance staff to enable them to handle the electronics in the new air conditioning and other changes in the plant.

In the United Kingdom the training system has historically been geared to initial training, taking place at the start of an individual's working life, through apprenticeships or higher education, and on and off the job training. However, new skills are needed not only by those just entering the labour market but also by those already in employment. Last month's *Employment* column showed how poorly the United Kingdom compared with its major competitors in relation to investment in such training.

A recent Institute of Manpower Studies (IMS) report¹ has shown the massive increase in skills training likely to be needed when industry does adopt the latest technology. The report, which looked at the adoption of new technology since 1980 in nearly 1,000 companies, shows that so

far the new technologies have had only a limited effect on the skills of employees, as there was still a very low take-up of new technology in areas such as research and development, and in office and administrative functions. While most companies had made some such investment in the production area, very few were yet able to draw on the potential of microelectronics technology in order to integrate several functions across the company, for example, by linking design with production and with sales and materials planning.

The impact on skills has been only limited so far because many employers have sought technology that could be handled by their existing workforce, or the changes involved were marginal or gradual. In addition, some employers had yet to think through the skills implications. The groups most likely to be affected were the technicians, supervisors and foremen, who often experienced an enhancement in status, and engineering craftsmen (Table 1). For these groups a key need was for multi-skilling, a pressure that was increased by recession-induced organizational change which sought to give the companies greater flexibility and reduce job demarcations. To obtain these new skills, the majority of companies had retrained their own staff, a course adopted by over two in three, while one in four sought to recruit such skills from the labour market, and a few used agency staff or temporaries (Table 2). For some groups of workers, especially those with job-specific skills, the new technologies led to de-skilling.

A separate IMS study² shows the experiences and lessons from ten organizations who have successfully adopted microelectronics and retrained the necessary staff. It highlights many of the issues to be resolved, including the identification of training needs, selection of trainees and resourcing the training. Perhaps most interestingly, this study shows how a small injection of training, very often involving courses of no more than five days' duration, provided major benefits. For individuals the returns included increased job satisfaction and improved job prospects, while it also gave them the flexibility and ability to cope with future change. The benefits for the employers included in one case the survival of the company, for others the rapid utilization of new technology, increased competitiveness and the release of high-level skills.

Finding people and organizations to do

the training was not a problem: on the job and off the job training in the company, equipment suppliers, private and public training organizations, colleges and distance learning were all used. Perhaps most interesting was the use by employers of a trade union, the EETPU, which has developed electronics training courses for electricians. These are run at its own training centre and via a mobile instructor service at employers' premises or in local union offices. The courses, which have been attended by over 2,000 trainees from over 200 sponsoring companies, are seen as highly successful because they are practical and geared to electricians who may not have received any formal training for ten years or more. The technology itself is now also becoming a major aid to training, especially computer- and video-based learning, which allows flexible training provision and can let trainees learn at their own speed and at times convenient to them.

The lessons of the case studies are that retraining, as well as improving skills and job performance, can boost employee confidence and commitment to change, and through job redesign increase the utilization of some higher-level skills and reduce

Table 2 Source of new skills

	Retrain	Recruit	Other
Technicians	64%	29%	7%
Draughtsmen	64%	23%	12%
Supervisors	91%	9%	—
Craftsmen	72%	22%	6%

Source, ref. 1.

job demarcations. For retraining to aid the successful introduction of new technology, it does, however, require that top management commitment and employee involvement should be there at the start, and a strategy developed to cope with the long-term need for retraining and continual change.

The costs of retraining need not be high, while the benefits accrue in both the short and the long term. A failure to invest in retraining and change by individuals, employers and nations is, however, a failure to invest in the future. □

1. *Training and Recruitment Effects of Technical Change* (Gower, Aldershot, 1985).
2. *Retraining for Electronics* (NEDO, London, 1985).

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Table 1 Impact of new technology on skills

	Not at all	Limited	Large
		extent	
Technician	17%	60%	23%
Draughtsmen	44%	46%	10%
Supervisors	13%	69%	18%
Craftsmen	18%	58%	24%

Source, ref. 1.